SOIL BIOASSAY PILOT STUDY

Evaluation of Screening Level Bioassays
for Use in Soil Toxicity Assessments at Hazardous Waste Sites
Under the Model Toxics Control Act

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ABSTRACT

The Washington State Department of Ecology is developing guidelines for addressing environmental protection at hazardous waste sites under the Model Toxics Control Act (MTCA). Five bioassay protocols being evaluated for use in conducting soil toxicity screening under MTCA were tested under a range of environmental conditions and contaminant concentrations. The five bioassays evaluated were 1) *Daphnia magna*- static acute 48 hour, 2) Plant Vigor (*Lactuca sativa*)- growth 14 day, 3) Earthworm (*Eisenia foetida*)- survival 14 day, 4) Fathead Minnow (*Pimephales promelas*)- static acute 48 hour, and 5) Frog Embryo Teratogenesis Assay Xenopus (FETAX), (*Xenopus laevis*)- whole embryo static renewal 96 hour. Soil contaminants included: heavy metals, petroleum products (gasoline and diesel), creosote, and pesticides.

Significant toxic responses were obtained with all five of the screening level bioassays. Overall the greatest number of toxic responses to the bioassay suite (percentage of samples tested that exhibited a toxic response for all bioassays) was measured at the creosote contaminated site (42%). Responses to the bioassay suite at the other sites were as follows; petroleum products (32%), metals (26%), and pesticides (13%). FETAX was the most sensitive bioassay tested (55% of the samples tested exhibited a toxic response).

Comparing the bioassay responses obtained to MTCA residential soil cleanup levels suggests that the established human health standards for the chemicals tested might not provide adequate protection for environmental concerns in several instances.

Recommendations for modifying the bioassay protocols are also provided.

INTRODUCTION

The Washington State Department of Ecology is in the process of developing guidelines for addressing environmental protection at hazardous waste sites under the Model Toxics Control Act (MTCA). Briefly, the proposed ecological assessment process involves using a tiered assessment approach to evaluate the toxicity of site soils and sediments. The cornerstone of tier-one involves screening site soils with a suite of bioassays (*Daphnia*, Earthworm, Plant Vigor, Fathead Minnow, and Frog Embryo Teratogenesis Assay Xenopus (FETAX)). These bioassays were selected to represent a range of ecological communities that could be affected by soil contamination at hazardous waste sites.

Presently, draft guidelines for conducting the tiered assessment process are being developed. In addition, draft protocols have also been prepared for conducting each of the tier-one bioassays. However, none of the bioassay procedures had been tested under actual field conditions. This evaluation was the focus of the Soil Bioassay Pilot Study.

Ecology's Toxics Cleanup Program contracted the Environmental Investigations and Laboratory Services Program to conduct the Soil Bioassay Pilot Study. The primary objectives of this study are listed below;

- Evaluate the toxicity of field-collected hazardous waste site soils with the five tier-one screening level bioassays.
- Identify potential problems with conducting the five bioassay procedures as currently written.
- Estimate the variability among replicates (a single sample that is homogenized and split into multiple aliquots) within each bioassay.

The results of this investigation will be used to modify the draft bioassay protocols. Once finalized these bioassay protocols may be used in developing guidance on evaluating hazardous waste site soils for potential toxicity to ecological communities.

METHODS

Site Selection

To be representative of a variety of environmental conditions in Washington State, the following criteria were used to select site types for sampling:

- Represent a range of contaminants frequently encountered at hazardous waste sites statewide; and
- provide an equal distribution of sites between Eastern and Western Washington.

Using these criteria and the available information on hazardous waste sites in Washington the following site types were selected for study.

I. Western Washington
   - Metals
   - Creosote
   - Petroleum Products (Leaking Underground Storage Tanks (LUST))

II. Eastern Washington
   - Metals
   - Pesticides
   - Petroleum Products (LUST)

Specific sites for each category were chosen based on recommendations by regional Ecology staff, availability of previous site characterization data, and logistical considerations. Preference was given to sites with shallow soil contamination, where the distribution of contaminants was fairly well known, and where there are some ecological concerns associated with the site. The general location of each site sampled is shown in Figure 1.

To test each bioassay over a range of contaminant concentrations, three separate locations were sampled at each site. The three locations represented relative areas of high, medium, and low (background) contaminant levels. Medium concentration samples were collected, to the extent possible, from locations with contaminant levels which approximated the MTCA residential soil cleanup standards (Ecology, 1991b). Table 1 lists detailed information on each sampling location.

Sampling Procedures

Site soils were collected with the use of either a hand-operated stainless steel bucket auger or alternately with a backhoe. Sampling equipment and soils collected are described in Appendix A, Table A1. At each site, duplicate (a single sample homogenized and split into two samples for analysis) soil samples were collected and prepared from each of the three sampling areas (high, medium, and low). Station positions were located with the use of a Magellan® NAV 5000D Global Positioning System (GPS) receiver.

At each station, an adequate amount of soil for duplicate analyses was collected and composited into a stainless steel bucket. The composite was then homogenized by stirring with a stainless steel spoon. Subsamples for specific analyses, with the exception of BTEX, were all taken from this homogenate. Aliquots for BTEX determinations were taken from the composite prior to homogenizing and placed directly in appropriate sample containers. To provide a more uniform grain size for the bioassay analyses, at several stations (see Appendix A, Table A1) samples were sieved prior to compositing. Immediately after collection the sample containers were wrapped in polyethylene bags and stored in coolers at 4°C for transport to the laboratory.

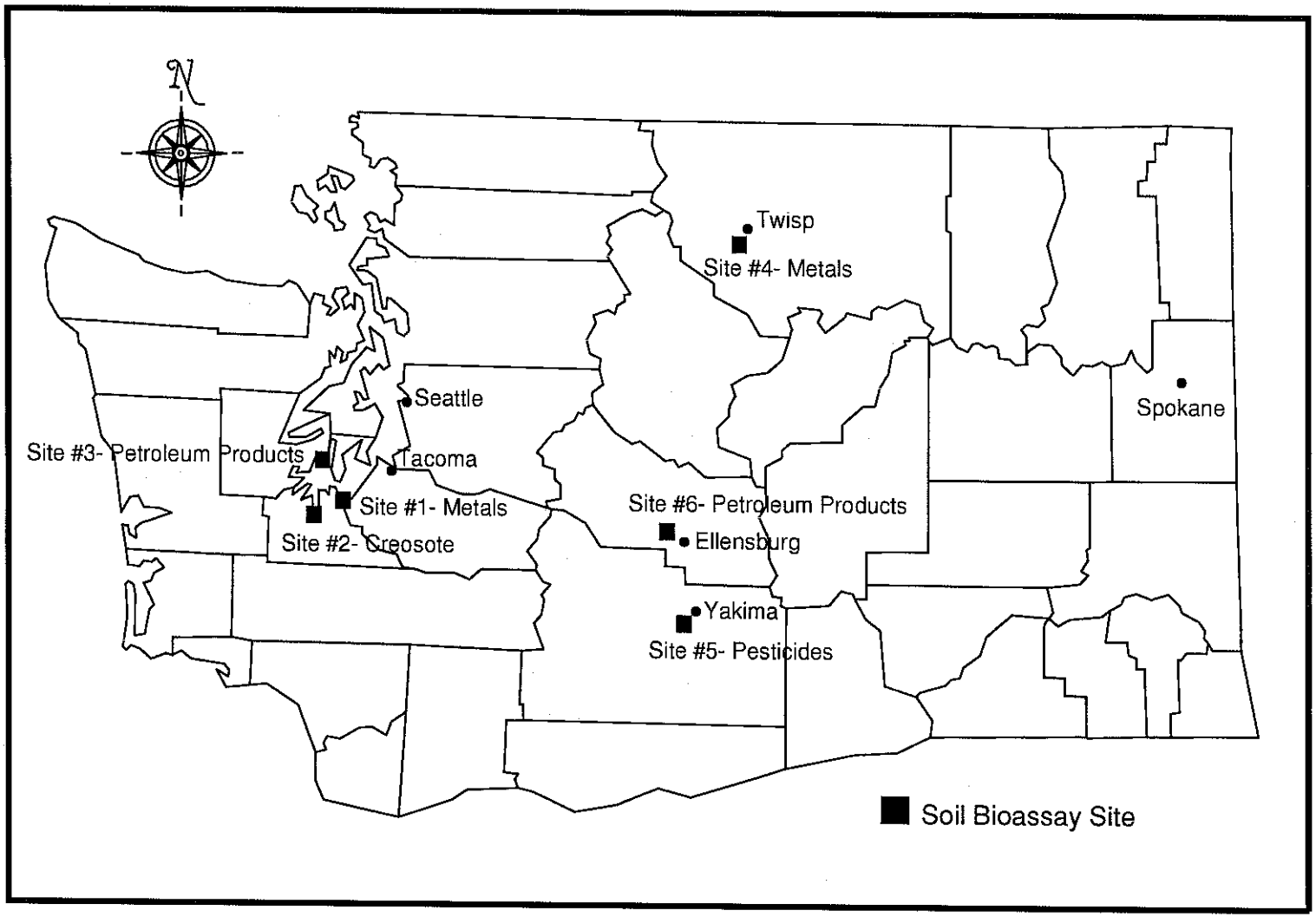

Figure 1: Locations of Sites Sampled for the Soil Bioassay Pilot Study.

Table 1: Station descriptions for the Soil Bioassay Pilot Study.

I. WESTERN WASHINGTON

Station	Latitude	Longitude	Elevation (ft)	Date Sampled	Sampling Interval (ft-bgs)	Conc. Range	Description
Site #1- Metals							
WD-1	47 5 59	122 39 53	270	2/8/93	0.25-1.0	Background	Clearcut at station BG-504
WD-2	47 6 37	122 39 42	210	2/8/93	0.25-0.5	Medium	Area 26 at station 26-TP-501
WD-3	47 6 39	122 39 45	270	2/8/93	0.25-0.5	High	Area 26 at station 26-HA-502
Site #2- Creosote							
CP-1	47 3 25	122 54 7	15	2/10/93	0.17-1.0	Background	Southwest corner of site 20' inside fence
CP-2	47 3 29	122 54 3	15	2/10/93	1.5-2.0	Medium	North of well EW-10 approx. 3'
CP-3	47 3 27	122 54 0	15	2/10/93	0.17-1.0	High	North of well EW-1 approx. 3'
Site #3- Petroleum Products							
JC-1	47 17 6	122 53 14	30	2/9/93	0.25-1.0	Background	North of excavation approx. 200'
JC-2	47 17 6	122 53 14	30	2/9/93	7.0-9.0	Medium	Middle of excavation at 8'
JC-3	47 17 6	122 53 14	30	2/9/93	7.0-9.0	High	South wall of excavation at 8'

II. EASTERN WASHINGTON

Station	Latitude	Longitude	Elevation (ft)	Date Sampled	Interval (ft-bgs)	Conc. Range	Description
Site #4- Metals							
AM-1	48 21 24	120 7 33	2100	3/3/93	1.0-3.0	Background	North of BM upgradient well approx. 2'
AM-2	48 21 18	120 7 27	2100	3/3/93	3.0-5.5	Medium	North of BM well P6 approx. 2'
AM-3	48 21 10	120 7 21	2100	3/3/93	3.0-5.5	High	North of BM well P2 approx. 2'
Site #5- Pesticides							
RA-1	46 33 35	120 32 5	800	3/2/93	1.0-3.0	Background	Pasture southwest of washdown area
RA-2	46 33 35	120 32 5	800	3/2/93	1.0-2.0	Medium	Southeast of site #1 marker approx. 5'
RA-3	46 33 35	120 32 5	800	3/2/93	1.0-2.5	High	Northwest of site #2 marker approx. 5'
Site #6- Petroleum Products							
B-1	47 3 11	120 39 56	1600	3/1/93	0.5-1.5	Background	Southeast of access road in field
B-2	47 3 11	120 39 56	1600	3/1/93	2.5-3.5	Medium- (Gasoline)	Adjacent to RI station S27
B-3	47 3 11	120 39 56	1600	3/1/93	2.5-3.5	Medium- (Diesel)	Base of above ground tanks in piping trench (S14
B-4	47 3 11	120 39 56	1600	3/1/93	5.5-6.5	High- (Gasoline)	Southeast wall of excavation 20' from road
B-5	47 3 11	120 39 56	1600	3/1/93	2.0-3.5	High- (Diesel)	Adjacent to RI station S19

Latitude/Longitude=Degrees/Minutes/Seconds

ft-bgs= Feet below ground surface

All sampling equipment was pre-cleaned with sequential washes of hot tap water/Liquinox® detergent, 10 percent nitric acid, distilled/deionized water, and pesticide grade acetone, then air-dried and wrapped in aluminum foil until used in the field. In addition, to minimize the potential for cross-contamination, samples were collected in order of increasing contaminant levels, based on previous data and reconnaissance work.

Analyses

To provide consistency in analytical procedures and to verify previous site characterization data, physical/chemical analyses were conducted in conjunction with the bioassays. The suite of chemical and biological tests performed on soils at each site is summarized in Table 2.

Chemical

Analytical methods and laboratories used in the Soil Bioassay Pilot Study are listed in Table 3. Quality of the chemical data was assessed by analysis of procedural blanks, matrix spikes, surrogate spikes, internal standards, and laboratory control samples.

Quality assurance/quality control (QA/QC) review of the data was performed by the following individuals at the Ecology/EPA Manchester Laboratory: David Thompson- conventionals; Bill Kammin- metals; and Karin Fedderson- organics, except semivolatiles which were reviewed by Dickey Huntamer. The data were reviewed for qualitative and quantitative accuracy, validity, and usefulness. No major analytical problems were encountered with the analysis of these samples. Consequently, the data are considered acceptable for use, with the accompanying qualifiers noted where appropriate. Case narratives and data reviews for the physical/chemical analyses are included in Appendix B. Unless otherwise noted all concentrations in this document are reported on a dry weight basis.

Bioassay

Five bioassays were conducted for the Soil Bioassay Pilot Study. They are as follows:

- *Daphnia magna*- Static acute 48 hour;
- Plant Vigor (*Lactuca sativa*)- Growth 14 day;
- Earthworm (*Eisenia foetida*)- Survival 14 day;
- Fathead Minnow (*Pimephales promelas*)- Static acute 48 hour; and
- Frog Embryo Teratogenesis Assay Xenopus (FETAX), (*Xenopus laevis*)- Whole embryo static renewal 96 hour.

Table 2: Summary of analyses by site for the Soil Bioassay Pilot Study.

I. WESTERN WASHINGTON

Site #1- Metals	Site #2- Creosote	Site #3- Petroleum Products
Percent Solids	Percent Solids	Percent Solids
Grain Size	Grain Size	Grain Size
TOC	TOC	TOC
Ammonia	Ammonia	Ammonia
Sulfide	Sulfide	Sulfide
PP Metals	WTPH-418.1	BTEX
Daphnia	Semivolatiles	WTPH-G
Earthworm	Daphnia	Semivolatiles
Plant Vigor	Earthworm	Daphnia
Fathead Minnow	Plant Vigor	Earthworm
FETAX	Fathead Minnow	Plant Vigor
	FETAX	Fathead Minnow
		FETAX

II. EASTERN WASHINGTON

Site #4- Metals	Site #5- Pesticides	Site #6- Petroleum Products
Percent Solids	Percent Solids	Percent Solids
Grain Size	Grain Size	Grain Size
TOC	TOC	TOC
Ammonia	Ammonia	Ammonia
Sulfide	Sulfide	Sulfide
Cyanide	Chlorinated Herb	BTEX
PP Metals	Chlorinated Pest	WTPH-G
Daphnia	Organophosphorus Pest	WTPH-D
Earthworm	Daphnia	Semivolatiles
Plant Vigor	Earthworm	Daphnia
Fathead Minnow	Plant Vigor	Earthworm
FETAX	Fathead Minnow	Plant Vigor
	FETAX	Fathead Minnow
		FETAX

PP Metals= Priority pollutant metals (see Table 3)

Table 3: Summary of analytical methods and laboratories utilized for the Soil Bioassay Pilot Study.

Analyte	Method	Reference	Laboratory
Conventionals			
Percent Solids	Dry @ 104°C	PSEP, 1986	Ecology/EPA Manchester Lab., Manchester, WA.
Grain Size	Seive and Pipet	"	Soil Technology, Inc., Winslow, WA.
TOC	Combustion/CO2 Measurement	"	Analytical Resources, Inc., Seattle, WA.
Ammonia	KCL Extraction	Plumb, 1981	" " " "
Sulfide	Spectrophotometric	PSEP, 1986	" " " "
Metals (Total)			
Hg	CVAA	EPA, 1986	Ecology/EPA Manchester Lab., Manchester, WA.
As,Se,and Tl	GFAA	"	" " " "
Sb,Be,Cd,Cr,Cu, Ni,Pb,Ag,and Zn	ICP	"	" " " "
Cyanide	Colorimetric-4500-CN	APHA, 1992	" " " "
Organics			
WTPH-418.1	GC/FID	Ecology, 1991a	" " " "
WTPH-G	GC/FID	"	Sound Analytical Services, Tacoma, WA. (February) Ecology/EPA Manchester Lab., Manchester, WA. (March)
WTPH-D	GC/FID	"	" " " "
BTEX	Purge and Trap #8020	EPA, 1986	Sound Analytical Services, Tacoma, WA. (February) Ecology/EPA Manchester Lab., Manchester, WA. (March)
Semivolatiles	GC/MS #8270	"	" " " "
Chlorinated Pest	GC/ECD #8080	"	Analytical Resources, Inc., Seattle, WA.
Organophosphorus Pest	GC/NPD #8140	"	" " " "
Chlorinated Herb	GC/ECD #8150	"	" " " "
Bioassays			
Daphnia	Static Acute 48-hr	Ecology, 1992a	Ecology/EPA Manchester Lab., Manchester, WA.
Fathead Minnow	Static Acute 48-hr	Ecology, 1992d	" " " "
Plant Vigor	Growth 14-day	Ecology, 1992b	Springborn Laboratories, Inc., Wareham, MA.
Earthworm	Survival 14-day	Ecology, 1992c	" " " "
FETAX	Static Renewal Whole Embryo 96-hr	Ecology, 1992e	Stover Biometric Laboratories, Inc., Stillwater, OK.

WTPH= Washington State modification of federal total petroleum hydrocarbons method.

Bioassay testing procedures followed draft protocols developed for conducting ecological assessments under MTCA (Ecology, 1992a,b,c,d,e). Laboratories performing each of the bioassays are listed in Table 2.

QA/QC review of the bioassay results was performed by Margaret Stinson of the Ecology/EPA Manchester Laboratory. QA/QC data were consistent with test acceptability requirements outlined in the bioassay protocols with the exception discussed below.

In the first set of Plant Vigor tests (Western Washington) lettuce seed germination was only 50% in the negative control. For test acceptability, 90% is required. Possible explanations for the low germination rate include poor seed viability and inadequate hydration during testing. During the second set of tests a new batch of seeds was used, resulting in adequate germination to meet acceptance criteria. Plant Vigor results from Site #1, #2, and #3 are reported but not used in calculations due to poor seed germination in the negative control.

Protocols for the Plant Vigor and Earthworm tests do not address rehydration of soils during testing. During testing it was necessary to rehydrate the samples to insure that the test organisms would survive until day 14 (see Appendix B, bioassay case narratives). Approximately 10-30 ml of deionized water was added to each replicate on day 7 of the tests.

During round one of sampling, an insufficient amount of sample for several of the sites was provided to the laboratory. As a result, to provide consistency among samples, test volumes in all samples were reduced in both the Plant Vigor (specified= 100 g, used= 80 g) and Earthworm (specified= 220 g, used= 175 g) tests. For consistency and comparability, similar test volumes were used for the second round of samples.

Finally, the protocol for the Earthworm bioassay specifies that the test organisms should weigh between 300 - 500 mg at initiation of the test. Mean weights of the *Eisenia foetida* used in this study were approximately 200 mg. This should not pose a problem for evaluating the data since the worms used were mature (*i.e.*, possessing a clitellium) and a smaller sample volume was also used. Consequently, the loading rate of test organisms to soil is similar to what is specified in the draft protocol.

Case narratives and QA/QC reviews for each of the bioassays are included in Appendix C. The reader is referred to this section of the document for a detailed explanation of the bioassay procedures used.

RESULTS

Conventionals

The results of conventionals analyses of soils from the six hazardous waste sites sampled for the Soil Bioassay Pilot Study are summarized in Table 4. Total solids ranged from 71 to

Table 4:Summary of conventionals analysis of soil samples collected in February and March, 1993 for the Soil Bioassay Pilot Study.

I. Western Washington

Location	Site #1			Site #2			Site #3		
Primary Contaminant	Metals			Creosote			Petroleum Products		
Station	WD-1	WD-2	WD-3	CP-1	CP-2	CP-3	JC-1	JC-2	JC-3
Conc. Range	Background	Medium	High	Background	Medium	High	Background	Medium	High
Total Solids (%)	88.3	77.9	84.2	94.5	86.8	86.4	92.3	83.6	79.8
TOC (%)	3.2	13	3.1	0.74	14	11	0.31	0.76	1.3
Ammonia (mg-N/kg)	2.3	1.7	21	0.59	2	3.8	2.7	1.4	1.5
Sulfide (mg/kg)	2.8 u	3 u	1.9 u	2.3 u	2.1 u	2.6 u	1.7 u	1.9 u	2.7 u
Grain Size (%)									
Gravel	0	0	22	11	19	12	46	16	30
Sand	93	81	50	83	68	81	43	47	32
Silt	5	13	19	3	8	4	6	26	23
Clay	2	6	9	3	5	3	5	11	15

I. Eastern Washington

Location	Site #4			Site #5			Site #6				
Primary Contaminant	Metals			Pesticides			Petroleum Products				
Station	AM-1	AM-2	AM-3	RA-1	RA-2	RA-3	B-1	B-2	B-3	B-4	B-5
Conc. Range	Background	Medium	High	Background	Medium	High	Background	Medium	Medium	High	High
Total Solids (%)	95.1	91.3	89.2	71	74.3	77.6	75.2	88	76.3	89.2	82.5
TOC (%)	0.5	0.05	0.06	1.2	0.61	0.69	2.4	0.39	2	0.72	1.1
Ammonia (mg-N/kg)	0.74	0.67	1.5	1.9	0.83	0.5	0.85	0.65	59	0.49	17.4
Sulfide (mg/kg)	2.1 u	2.5 u	2.5 u	3 u	3.3 u	3 u	3 u	1.8 u	3.3 u	1.5 u	2.2 u
Cyanide (mg/kg)	0.08	0.06	0.14	NA	NA	NA	NA	NA	NA	NA	NA
Grain Size (%)											
Gravel	34	0	0	1	7	0	1	39	2	34	10
Sand	34	55	57	27	42	33	38	46	39	54	29
Silt	26	39	37	57	45	53	42	10	42	7	38
Clay	6	6	6	15	6	14	19	5	17	5	23

u=Not detected at detection limit shown

NA=Not Analyzed

94.5%. Total organic carbon (TOC) was quite variable between sites ranging over 4 orders of magnitude (0.05 to 14%). The highest TOC levels were typically present at the creosote contaminated areas of Site #2, while the lowest were measured at Site #4 (metals). Ammonia concentrations were also quite variable ranging over two orders of magnitude (0.5 to 59 mg/kg). The highest ammonia levels were associated with diesel contaminated areas at Site #6. Sulfide was not detected in any of the samples tested. Cyanide concentrations at Site #4 were low ranging from 0.06 to 0.14 mg/kg.

The grain size distribution of site soils is shown in Figure 2. Most samples consisted of sand (2mm - 62m) and gravel (>2mm) size particles. In general, the grain size distribution within each site was fairly similar with the exception of Site #6 (petroleum products). At Site #6 two of the areas (B2 and B4) tended to have a higher percentage of gravel compared to the rest of the locations at Site #6. It should be noted that the grain size distributions shown do not reflect the actual site conditions for those samples that were sieved in the field. The reader is referred to Appendix A, Table A1 for a list of stations that were sieved in the field.

Metals (Site #1 and #4)

Metals concentrations in soils from Sites #1 and #4 are listed in Table 5. Metals concentration at Site #1 were generally low with the exceptions of the primary contaminants of concern at this site, lead and mercury. Lead concentrations ranged from 8.8 to 110,000 mg/kg. Mercury concentrations ranged from 0.04 to 56 mg/kg. At Site #4 the primary contaminants of concern were copper and zinc. Copper concentrations ranged from 58 to 1,400 mg/kg, while zinc levels ranged from 85 to 990 mg/kg. Arsenic was also present at somewhat elevated levels (45 - 84 mg/kg), however, not much of a concentration gradient was present between the three locations sampled.

Organics (Site #2, #3, #5, #6)

The results of total petroleum hydrocarbons (TPH) and semivolatile organics analysis of soils from Site #2 are summarized in Table 6. The primary contaminants of concern at Site #2 were TPH and carcinogenic PAHs (CPAH). CPAH includes the sum of the following compounds: benzo(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, chrysene, dibenzo(a,h)anthracene, and ideno(1,2,3-cd)pyrene. TPH and CPAH were not detected at the background station with the following detection limits (TPH= 38,000 ug/kg and CPAH= 340 ug/kg). The highest TPH (6,900,000 ug/kg) and CPAH (860,000 ug/kg) concentrations were present at station CP-3. Relatively high concentrations of pentachlorophenol (64,000 ug/kg) were also present at station CP-3.

In addition to the target compounds, sixty-two semivolatile organic compounds were also tentatively identified (TI) in soils at Site #2. TI compounds are found during mass spectral searches of the sample extracts; they represent some of the most prevalent peaks in sample chromatograms that were not among the original target compounds (PSEP, 1988). These

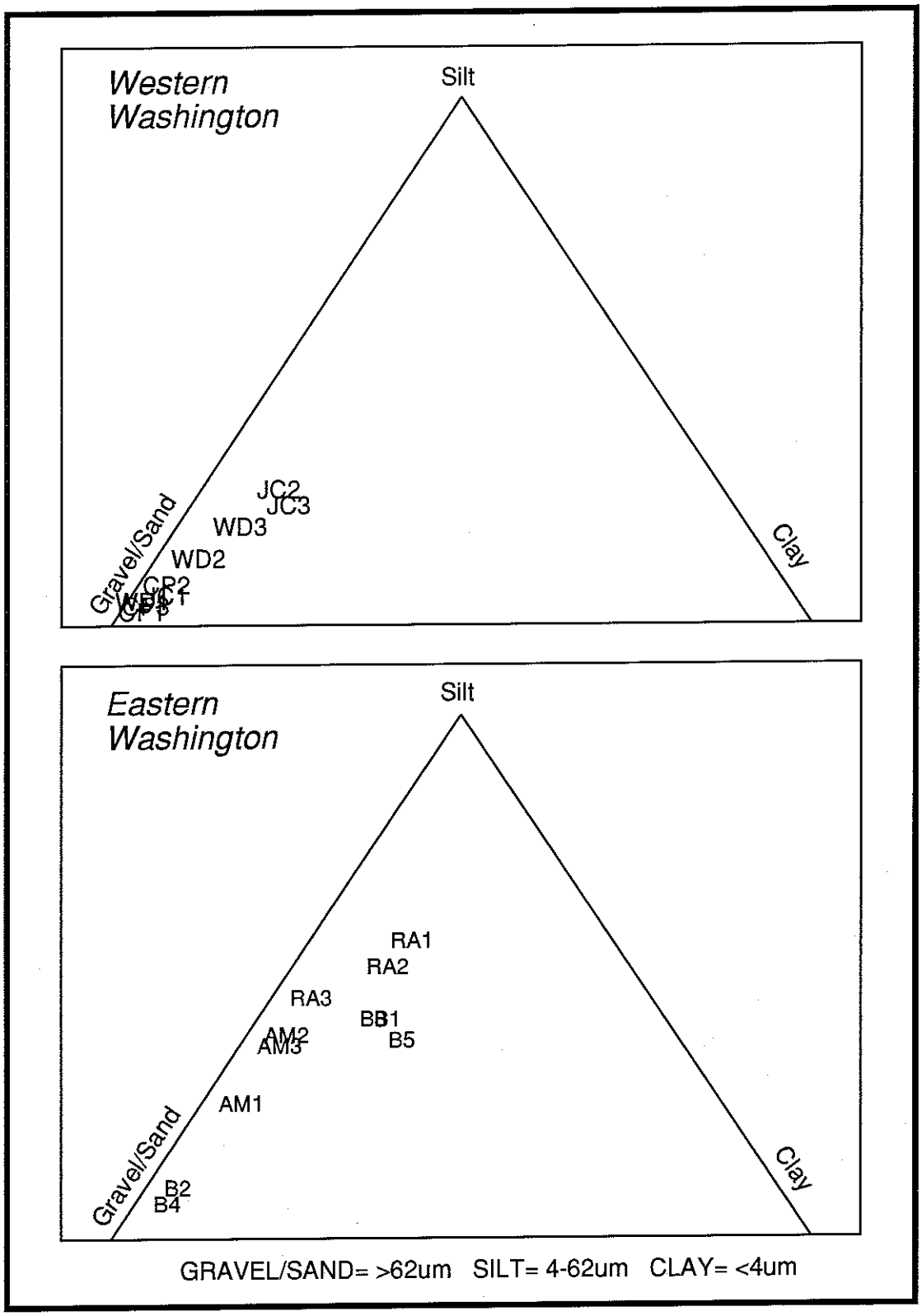

Figure 2:Grain size composition of soils collected from Western and Eastern Washington for the Soil Bioassay Pilot Study.

Table 5: Results of metals analysis of soils from sites #1 and #4 for the Soil Bioassay Pilot Study (mg/kg, dry).

Location	Western Washington Site #1			Eastern Washington Site #4		
Station	WD-1	WD-2	WD-3	AM-1	AM-2	AM-3
Conc. Range	Background	Medium	High	Background	Medium	High
Arsenic	8	25	7.9	84 j	46 j	45
Antimony	3 uj	3 uj	3 uj	3 uj	3 uj	3 uj
Beryllium	0.25 p	0.12 p	0.1 u	0.38 p	0.1 u	0.19 p
Cadmium	0.59 p	0.62 p	0.2 u	0.68 p	0.85 p	5.9
Chromium	17	17	3.3	12 j	1.3 pj	7.9 j
Copper	15	25	9.6	58	260	1400
Lead	8.8 p	490	110000	12 p	240	140
Mercury	0.04 p	1.4	56	0.01 p	0.9	0.35
Nickel	23	15	1.8 p	10 j	2.5 pj	2.4 pj
Selenium	0.4 u	1.7	0.4 u	0.4 u	1.5	2.5
Silver	0.3 u	0.3 u	0.61 p	0.3 u	4.6	2.1 p
Thallium	0.5 u	0.5 u	0.5 u	0.5 u	1.6	0.57
Zinc	39	39	8.8 b	85	130	990

u=Not detected at detection limit shown

j=Estimated value

p=Analyte was detected above the instrument detection limit but below the established minimum quantitation limit

b=Analyte was also present in associated method blank at significant levels

Table 6: Summary of TPH and semivolatile organics analysis of soils from site #2 (creosote) for the Soil Bioassay Pilot Study (ug/kg, dry).

Location	Western Washington Site #2		
Station	CP-1	CP-2	CP-3
Conc. Range	Background	Medium	High
WTPH-418.1	38000 u	120000	6900000 j
Semivolatiles			
Acenaphthene	340 u	660	1500000
Acenaphthylene	340 u	290 j	42000
Naphthalene	340 u	11000	380000
Fluorene	340 u	530	5200000
Anthracene	340 u	1400	970000
Phenanthrene	340 u	2100	2600000
Sum LPAH	-	16000 j	11000000
Fluoranthene	340 u	4700	2300000
Benzo(a)anthracene*	340 u	1600	290000
Chrysene*	340 u	2700	270000
Pyrene	340 u	4900	1400000
Benzo(b)fluoranthene*	340 u	2400	140000
Benzo(k)fluoranthene*	340 u	850	56000
Benzo(a)pyrene*	340 u	1300	74000
Dibenzo(a,h)anthracene*	340 u	180 j	7200 j
Indeno(1,2,3-cd)pyrene*	340 u	790	25000
Benzo(g,h,i)perylene	340 u	500 u	15000 j
Sum HPAH	-	19000 j	4600000 j
Sum CPAH	-	9800 j	860000 j
1-Methylnaphthalene	340 u	620	480000 j
2-Methylnaphthalene	340 u	760	410000
Dibenzofuran	340 u	450	560000
Carbazole	340 u	210 j	90000
Pentachlorophenol	3400 u	390 u	64000 j
Bis(2-ethyl hexyl) phthalate	340 u	390 u	16000 j

u=Not detected at detection limit shown

j=Estimated concentration

*CPAH= Carcinogenic Polynuclear aromatic hydrocarbons

compounds, listed in Appendix D, Table D1, were primarily alkyl substituted PAHs (i.e. an alkyl group has been substituted for a hydrogen on one or more of the aromatic rings. Some of the more common alkyl groups are; ethyl-, methyl-, isopropyl-, and butyl-). The greatest number of TI compounds were present at station CP-3.

Summarized in Table 7 are benzene, toluene, ethylbenzene, and xylene (BTEX); TPH, and semivolatile organics detected in soils at Sites #3 and #6. At Site #3 BTEX concentrations ranged from not detected at 40 ug/kg to 1,000,000 ug/kg. TPH-G (gasoline) was not detected at the background station. At JC-3 TPH-G concentrations peaked at 6,900,000 ug/kg. The high concentrations of BTEX range hydrocarbons in these soils indicates that the petroleum contamination is recent.

No BTEX range hydrocarbons were detected at Site #6. TPH-G was only present at station B-2. The gasoline at station B-2 was extremely weathered and had lost the entire BTEX range of hydrocarbons. TPH-D (diesel) was measured at stations B-3 and B-5, at concentrations of 340,000 ug/kg and 6,200,000 ug/kg, respectively. The diesel oil at station B-2 was extremely weathered to the point where all of the major straight chain hydrocarbons had been lost. The diesel oil at station B-5, still contained the major straight chained hydrocarbons, but at smaller quantities than normal in unweathered diesel oil (Carrell, 1993). These data indicate that the diesel oil at station B-5 is a more recent addition to the environment than the diesel at station B-3.

Forty-two TI organics were also present in soils at Site #3 (Table D2). The most prevalent compounds were alkyl substituted benzenes. Several alkyl substituted naphthalenes were also present. At Site #6 twenty-five TI organics were detected. In contrast to Site #3, fewer of the more volatile benzenes were present. Alkyl substituted naphthalenes were the most prevalent group of compounds found. Several straight chain hydrocarbons were detected at both sites.

Results of soil analyses for pesticides and herbicides from Site #5 are summarized in Table 8. Five chlorinated pesticides were detected at Site #5. They included: aldrin, endosulfan I/II, dieldrin, DDT/DDE, and toxaphene. Toxaphene was present at the highest concentrations, with a peak concentration of 630 ug/kg. Maximum concentrations for aldrin and dieldrin were 110 ug/kg and 120 ug/kg, respectively. No organophosphorus pesticides or chlorinated herbicides were detected despite relatively low detection limits.

Bioassay

Table 9 summarizes the results of soil bioassay testing at each of the six hazardous waste sites. A complete listing of all bioassay results is included in Appendix C, case narratives and Tables C1-C5. For comparability in this report, determination of a significant toxic response in <u>all</u> bioassays was made by comparing the sample response to the negative control response using a one-tailed Dunnetts Test at $P < 0.05$ (Dunnett, 1955).

Table 7: Summary of BTEX, TPH and semivolatile organics analyses of soils from petroleum products sites #3 and #6 for the Soil Bioassay Pilot Study (ug/kg, dry).

Location	Western Washington Site #3			Eastern Washington Site #6				
Station	JC-1	JC-2	JC-3	B-1	B-2	B-3	B-4	B-5
Conc. Range	Background	Medium	High	Background	Medium (Gas)	Medium (Diesel)	High (Gas)	High (Diesel)
Benzene	40 uj	50 uj	3000 uj	25 u	21 u	–	20 u	–
Toluene	40 uj	170 j	160000 j	25 u	21 u	–	20 u	–
Ethyl benzene	40 uj	80 j	51000 j	25 u	21 u	–	20 u	–
Xylenes	40 uj	2300 j	800000	74 u	64 u	–	59 u	–
Sum BTEX	nd	2600 j	1000000	nd	nd	–	nd	–
TPH-G	1800 u	200000	6900000	1500 u	1100 j	–	1500 u	–
TPH-D	–	–	–	3500 u	–	340000	–	6200000
Semivolatiles								
Naphthalene	160 u	370 j	39000	210 u	180 u	230 u	200 u	380 j
Fluorene	160 u	280 u	420 u	210 u	180 u	230 u	200 u	740
Phenanthrene	160 u	280 u	390 j	210 u	180 u	230 u	200 u	1400
1-Methyl naphthalene	160 u	2000	33000	31 j	160 j	320 j	200 u	3700
2-Methyl naphthalene	160 u	1300	38000	21 j	130 j	520 j	200 u	1700
Di-n-butyl phthalate	160 u	280 u	420 u	210 u	210 j	230 u	200 u	340 u
Retene	160 u	280 u	420 u	140 j	180 u	230 u	200 u	340 u

u=Not detected at detection limit shown

j=Estimated concentration

–=Not analyzed

nd=No compounds detected

Table 8: Summary of pesticide and herbicide analyses of soils from site #5 for the Soil Bioassay Pilot Study (ug/kg, dry).

Location	Eastern Washington Site #5		
Station	RA-1	RA-2	RA-3
Conc. Range	Background	Medium	High
Chlorinated Pesticides			
Aldrin	3.2 u	3.4 u	110
Endosulfan I	3.2 u	3.3 j	80
Endosulfan II	6.3 u	6.7 u	65
Dieldrin	6.3 u	13 j	120
4,4' DDE	5.7	6.6	26 j
4,4' DDT	36	10 j	130
Toxaphene	320 u	320 j	630 j
Organophosphorus Pesticides	ND	ND	ND
Herbicides	ND	ND	ND

u=Not detected at detection limit shown

ND=No compounds were detected above quantitation limits

Table 9: Summary of toxicity testing results of soil samples collected in February and March, 1993 for the Soil Bioassay Pilot Study.

Western Washington

Location		Site #1			Site #2			Site #3		
Primary Contaminant		Metals			Creosote			Petroleum Products		
Station	WD-1	WD-2	WD-3	CP-1	CP-2	CP-3	JC-1	JC-2	JC-3
Conc. Range	Background	Medium	High	Background	Medium	High	Background	Medium	High
Daphnia magna (6 replicates)									
Survival (%)	97	100	0	92	100	0	93	83	0
Plant Vigor (6 replicates)									
Biomass (mg)	(11)	(16)	(11)	(14)	(6)	(2)	(33)	(8)	(15)
Germination (%)	(31)	(30)	(34)	(20)	(10)	(6)	(26)	(18)	(29)
Survival (%)	(31)	(30)	(34)	(20)	(10)	(6)	(26)	(18)	(29)
Earthworm (6 replicates)									
Survival (%)									
Day 7-	100	100	0	98	100	0	97	92	0
Day 14-	100	100	0	97	98	0	82	90	0
Sublethal Effects (Total no. individuals)									
Day 7-	100 L	100 L	-	94 L	100 L	-	7 L,LB	48 L	-
Day 14-	3 L	57 L	-	3 L	51 L	-	45 L	52 L	-
Fathead Minnow (3 replicates)									
Survival (%)	100	100	100	100	100	0	100	97	37
FETAX (3 replicates)									
Survival (%)	97	71	51	91	56	0	100	71	49
Malformation (%)	0	55	100	2.9	57	-	1.2	26	42
Mean Growth (mm)	85.3	87.4	73.4	85.8	87.9	-	88.7	86.4	85.7

Replicates= Number of replicates per sample

( )= Seed germination in control did not meet acceptance criteria of >90%.

Sublethal Effects codes for Earthworm test

L= Lethargic

LB= Lack of burrowing

[ ]=Statistically significant reduction in response relative to negative control (Dunnetts test-one tailed at P<0.05)

Table 9 (continued): Summary of toxicity testing results of soil samples collected in in February and March, 1993 for the Soil Bioassay Pilot Study.

Eastern Washington

Location		Site #4			Site #5			Site #6				
Primary Contaminant		Metals			Pesticides			Petroleum Products				
Station	AM-1	AM-2	AM-3	RA-1	RA-2	RA-3	B-1	B-2	B-3	B-4	B-5
Conc. Range	Background	Medium	High	Background	Medium	High	Background	Medium (Gas)	Medium (Diesel)	High (Gas)	High (Diesel)
Daphnia magna (6 replicates)											
Survival (%)	100	82	10	97	100	98	100	100	95	98	57
Plant Vigor (6 replicates)											
Biomass (mg)	95	110	130	84	26	9	68	88	52	56	16
Germination (%)	99	88	95	93	44	6	97	88	73	81	38
Survival (%)	99	88	95	93	44	6	97	88	73	81	38
Earthworm (6 replicates)											
Survival (%)											
Day 7-	98	100	95	100	98	100	98	100	100	97	22
Day 14-	93	100	93	100	95	98	98	100	100	97	22
Sublethal Effects (Total no. individuals)											
Day 7-	-	42 L	90 L	-	43 L	-	2 L	-	-	28 L	89 L
Day 14-	31 L	89 L	100 L	-	61 L	46 L	9 L	2 L	-	-	-
Fathead Minnow (3 replicates)											
Survival (%)	97	97	93	100	97	93	100	100	100	100	0
FETAX (3 replicates)											
Survival (%)	95	59	47	100	100	100	100	100	75	51	20
Malformation (%)	4.3	59	47	1.4	70	26	0	19	77	100	100
Mean Growth (mm)	87.3	64.6	78.3	87.2	88.5	88.3	86.0	88.0	85.5	88.1	68.7

Replicates= Number of replicates per sample

Sublethal Effects codes for Earthworm test

L= Lethargic

LB= Lack of burrowing

[ ] =Statistically significant reduction in response relative to negative control (Dunnetts test-one tailed at $P<0.05$)

Daphnia magna, Fathead Minnow, and FETAX are all short-term (48-96 hour) tests which evaluate the toxicity of aqueous sample extracts. In contrast, the Plant Vigor and Earthworm procedures are longer-term (14 day) solid phase evaluations.

As anticipated, in most instances toxic responses in all of the bioassays increased with higher contaminant concentrations (*i.e.*, dose response). All bioassays exhibited a toxic response at one or more of the sites tested. No significant responses were noted at any of the background stations. In most cases significant toxicity was observed at all stations with the highest contaminant levels. Exceptions follow: Fathead Minnow- Sites #1 (metals), #4 (metals), and #5 (pesticides); Earthworm- Site #4 and #5; and FETAX- Site #5. For these sites no significant toxic responses were measured with the bioassays listed. Plant Vigor was the only test that showed a toxic endpoint to soils from Site #5. For the majority of Sites FETAX was the only bioassay that consistently showed a significant toxic response at the intermediate concentration levels. Interestingly, at Site #4 biomass in the Plant Vigor test increased with increasing contaminant levels. This response sometimes indicates exposure to potentially toxic agents (hormesis). For the purposes of the present study, this was not considered to be a toxic response. As previously noted Plant Vigor results for Sites #1, #2, and #3 were invalidated due to poor seed germination in the negative control (see analysis section).

For information purposes, sublethal effects were also recorded in both the Earthworm and FETAX tests. Both of these tests indicated sublethal effects at a minimum of one station from all sites. Worm lethargy worms was the predominate sublethal effect observed in the Earthworm test. The lethargy of worms observed in the background samples at Site #1, #2, and #3 are most likely the result of the lack of moisture in these samples. In the FETAX test the greatest degree of teratogenic potential (*i.e.*, separation between mortality and malformation response rates) was observed at Site #1. The laboratory reported that samples from Sites #1 and #4 (metals contamination) both displayed malformations characteristically induced by exposure of *Xenopus* to several heavy metal mixtures. (See FETAX case narrative in Appendix B.)

DISCUSSION

Bioassay Variability

One of the major objectives of the Soil Bioassay Pilot Study was to evaluate the variability among replicates (a single sample homogenized and split into multiple aliquots) for a single treatment in each of the screening level bioassays. Shown below in Table 10 is a summary of the overall (all sites) variability among replicates (based on survival and biomass) for each of the bioassays. In addition, also shown is a summary of significant toxic responses (i.e., Hit percent = [no. of significant toxic responses/no. of samples] X 100) for each bioassay.

Table 10: Summary of variability among replicates and hit frequency for the Five Screening Level Bioassays.

Bioassay	S	C.V.	RPD	N	Hit Frequency	Hit Percent
Daphnia magna (percent survival)	6.3	0.04	19	120	5/20	25
Plant Vigor* (biomass)	17	0.61	160	117	3/11	27
Earthworm (percent survival)	5.3	0.13	31	117	4/20	20
Fathead Minnow (percent survival)	4.2	0.08	15	60	3/20	15
FETAX (percent survival)	3.8	0.08	16	60	11/20	55

S = Standard deviation of the sample
C.V. = Coefficient of variation
RPD = Relative percent difference (range of response/mean response)
N = Total number of replicates tested
Hit freq. = Based on mean response of all replicates (No. hits/No. of samples)
* = Includes Site #4, #5, and #6 only

These data indicate that *Daphnia*, Fathead Minnow, and FETAX had the lowest variability among replicates (RPD <20%). Slightly higher variability was measured for the Earthworm test (RPD= 31%). By far the highest variability was associated with the Plant Vigor test (RPD= 160%).

Overall the greatest number of toxic responses to the bioassay suite (percentage of samples tested that exhibited a toxic reponse for all bioassays) was measured at the creosote contaminated site (42%). Responses to the bioassay suite at the other sites were as follows; petroleum products (32%), metals (26%), and pesticides (13%).

For individual bioassays the highest hit percentage was measured for FETAX (55%). *Daphnia* and the Plant Vigor had similar hit frequencies of 25% and 27%, respectively. The lowest hit percentages were measured for Earthworm (20%) and Fathead Minnow (15%).

Comparison of Bioassay Response to Established MTCA Cleanup Levels

Currently, MTCA soil cleanup standards have been established for the protection of human health. The extent to which these cleanup levels also protect ecological communities is not

well understood. Figures 3-8 compare the responses (at test termination) of the five tier-one screening level bioassays to the MTCA cleanup levels for residential soils. It should be noted that these comparisons are based on a limited number of concentrations (3), and they do not take into account additive effects of multiple contaminants. Consequently, they are not intended to establish environmentally protective cleanup levels.

No bioassay hits were observed below the MTCA cleanup levels listed at Sites #1 (lead and mercury), #2 (CPAH), and #6 (TPH-D). At Site #2 (TPH) and Site #3 (BTEX and TPH-G) a toxic response was indicated in FETAX below the Method A cleanup levels for the compounds shown. Site #4 had significant toxic responses in both the Daphnia and FETAX tests below the Method B cleanup levels for copper and zinc. Plant Vigor was the only test which exhibited a toxic response below the cleanup levels for dieldrin and toxaphene at Site #5. These data suggest that in several cases the established MTCA human health cleanup levels may not provide adequate protection for some species in the ecological community. This points to the need to conduct toxicity testing at sites where environmental concerns are present.

CONCLUSIONS AND SUMMARY

Significant toxic responses were obtained under a range of environmental conditions and contaminate concentrations with all five of the tier-one screening level bioassays. The lowest toxicity was observed at the pesticides contaminated site where only 13% of the samples exhibited a toxic response to the bioassay suite. FETAX was the most sensitive bioassay tested. Comparing the bioassay responses obtained to MTCA residential soil cleanup levels indicates that the established human health standards for the chemicals tested might not provide adequate protection for environmental concerns in several instances.

The major findings of the Soil Bioassay Pilot Study are summarized below:

- In most instances toxic responses in all of the bioassays increased with higher contaminant concentrations (*i.e.*, dose response). Overall the greatest number of toxic responses to the bioassay suite (percentage of samples tested that exhibited a toxic response for all bioassays) was measured at the creosote contaminated site (42%). Responses to the bioassay suite at the other sites were as follows; petroleum products (32%), metals (26%), and pesticides (13%).

- For individual bioassays, the greatest number of significant toxic responses were measured with the FETAX (55%). *Daphnia* and Plant Vigor had similar hit frequencies (*Daphnia*= 25% and Plant Vigor= 27%). The fewest number of hits was measured for the Earthworm (20%) and Fathead Minnow tests (15%).

- *Daphnia*, Fathead Minnow, and FETAX had the lowest variability among replicates for all sites with RPDs (range of responses/mean response) of <20%. Slightly higher variability was measured for the Earthworm test (RPD= 31%). By far the highest variability was associated with the Plant Vigor test (RPD= 160%).

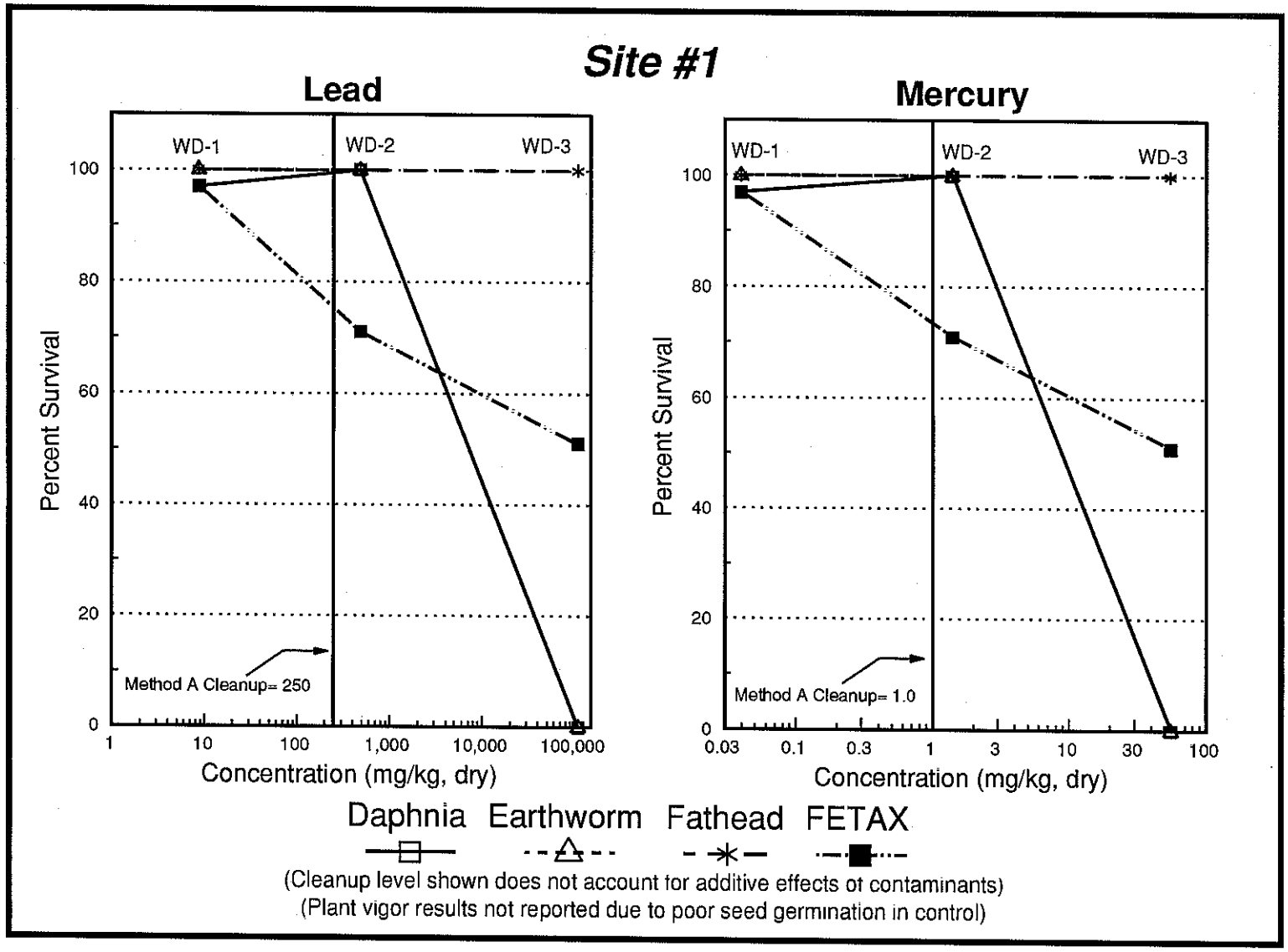

Figure 3:Response of bioassay suite to primary contaminants in soils at Site #1.

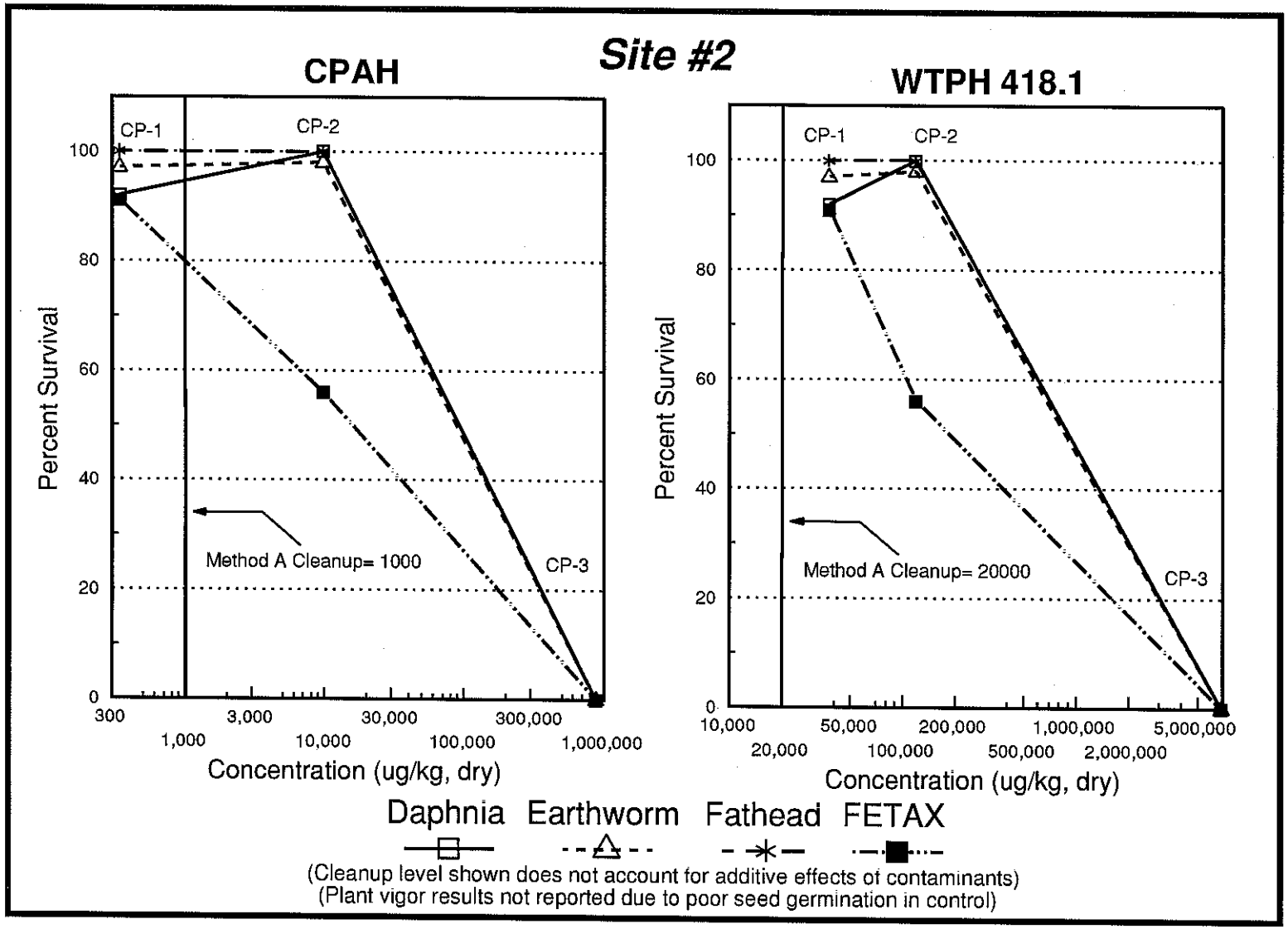

Figure 4:Response of bioassay suite to primary contaminants in soils at Site #2.

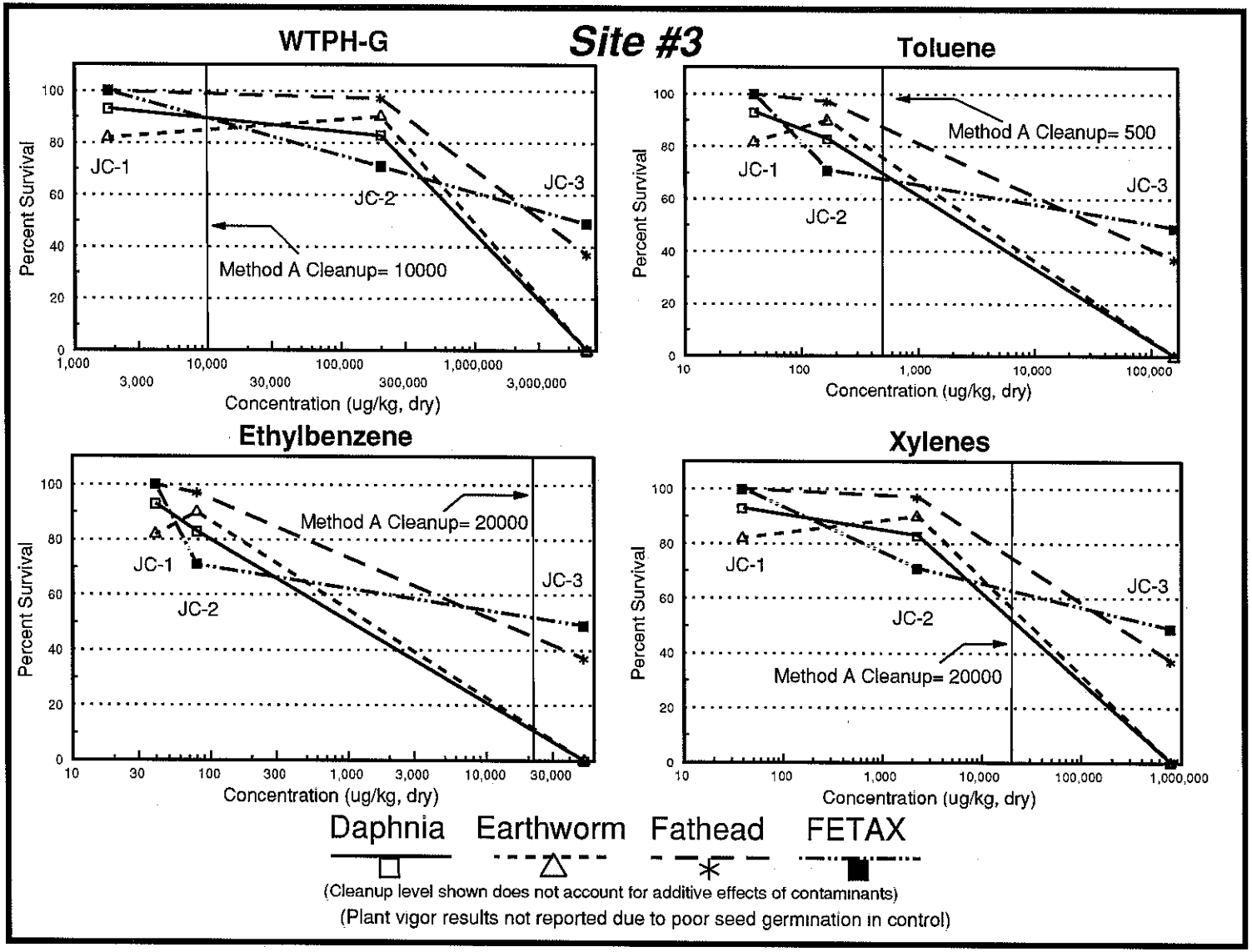

Figure 5:Response of bioassay suite to primary contaminants in soils at Site #3.

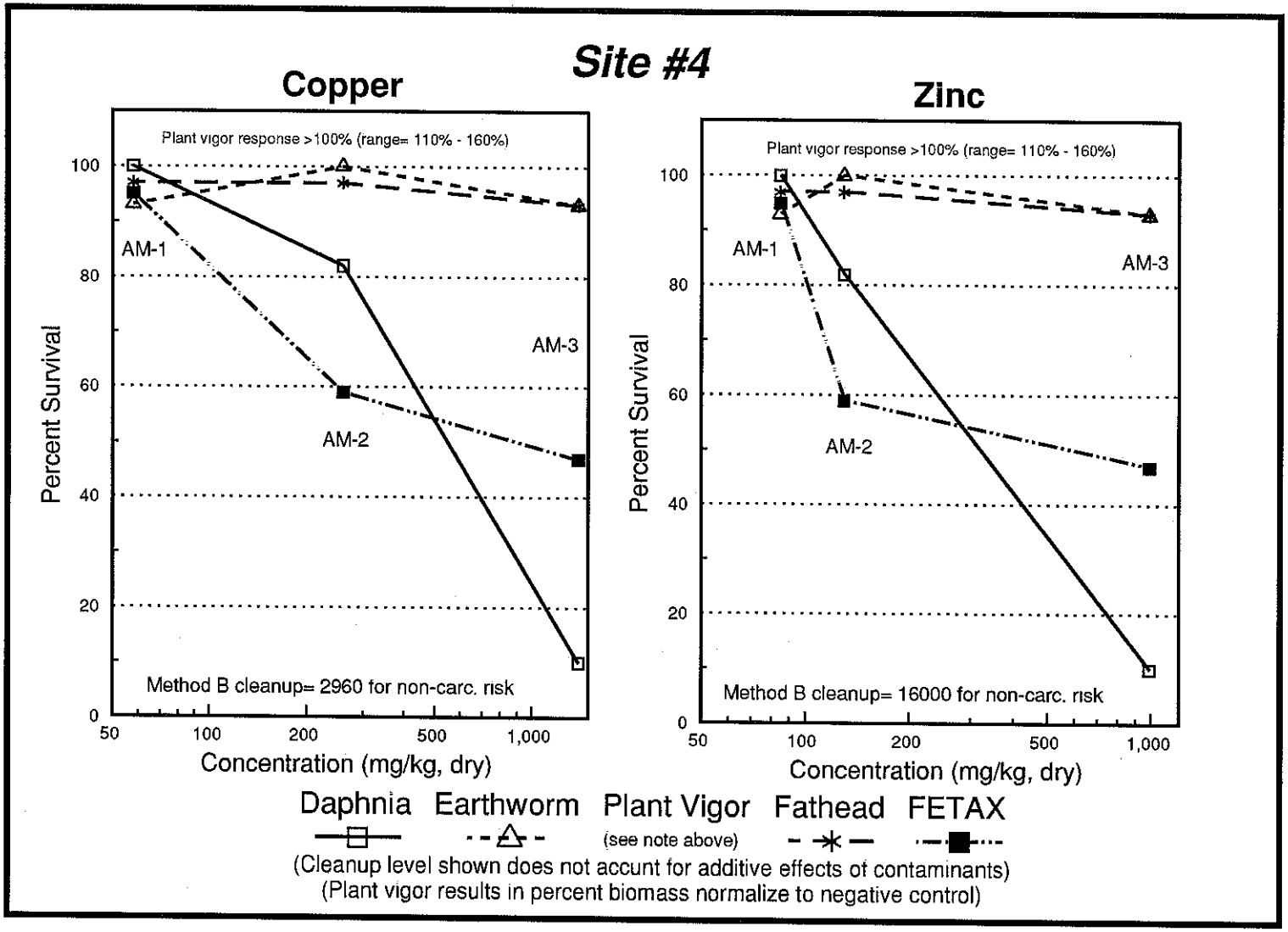

Figure 6:Response of bioassay suite to primary contaminants in soils at Site #4.

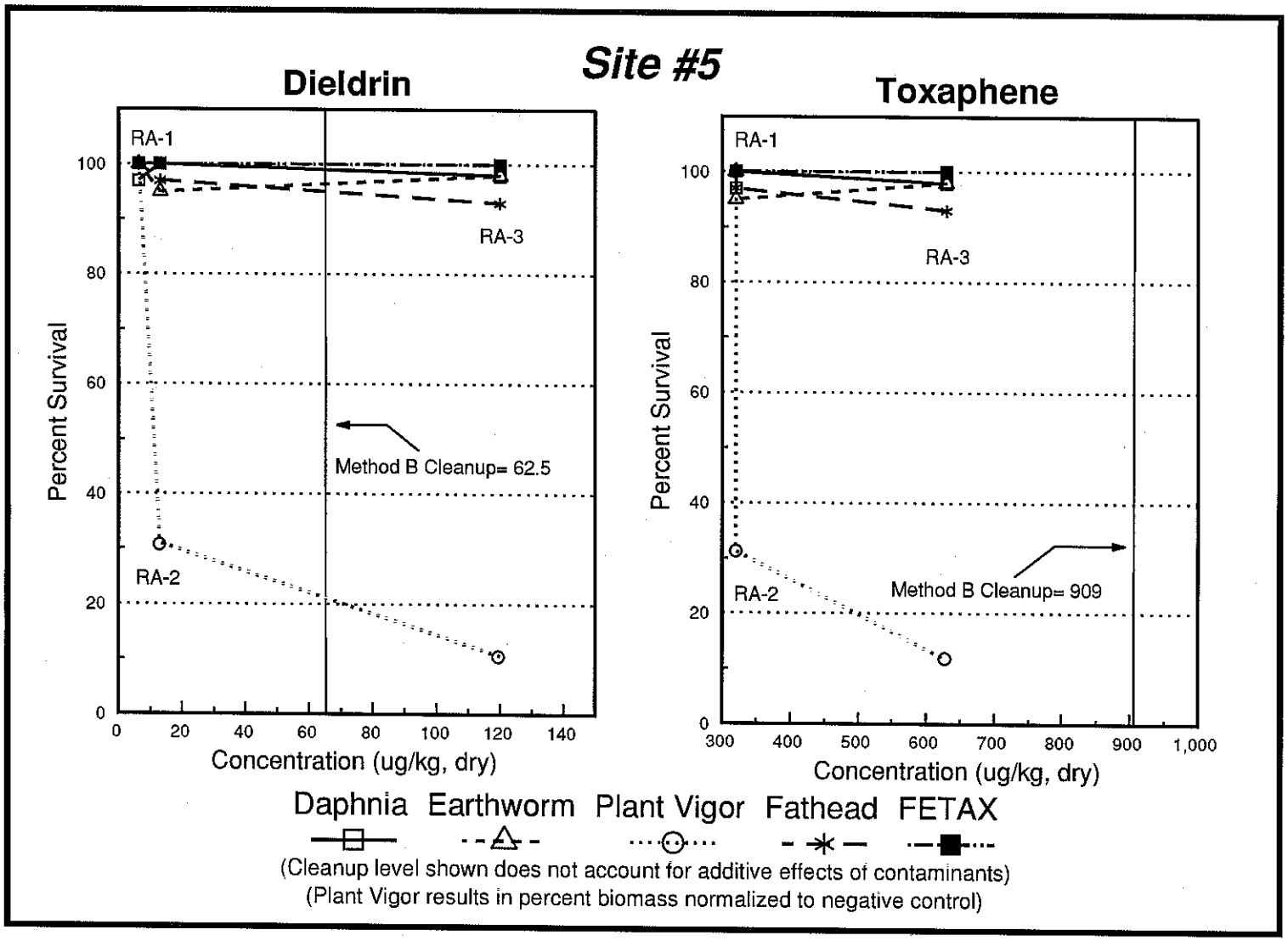

Figure 7:Response of bioassay suite to primary contaminants in soils at Site #5.

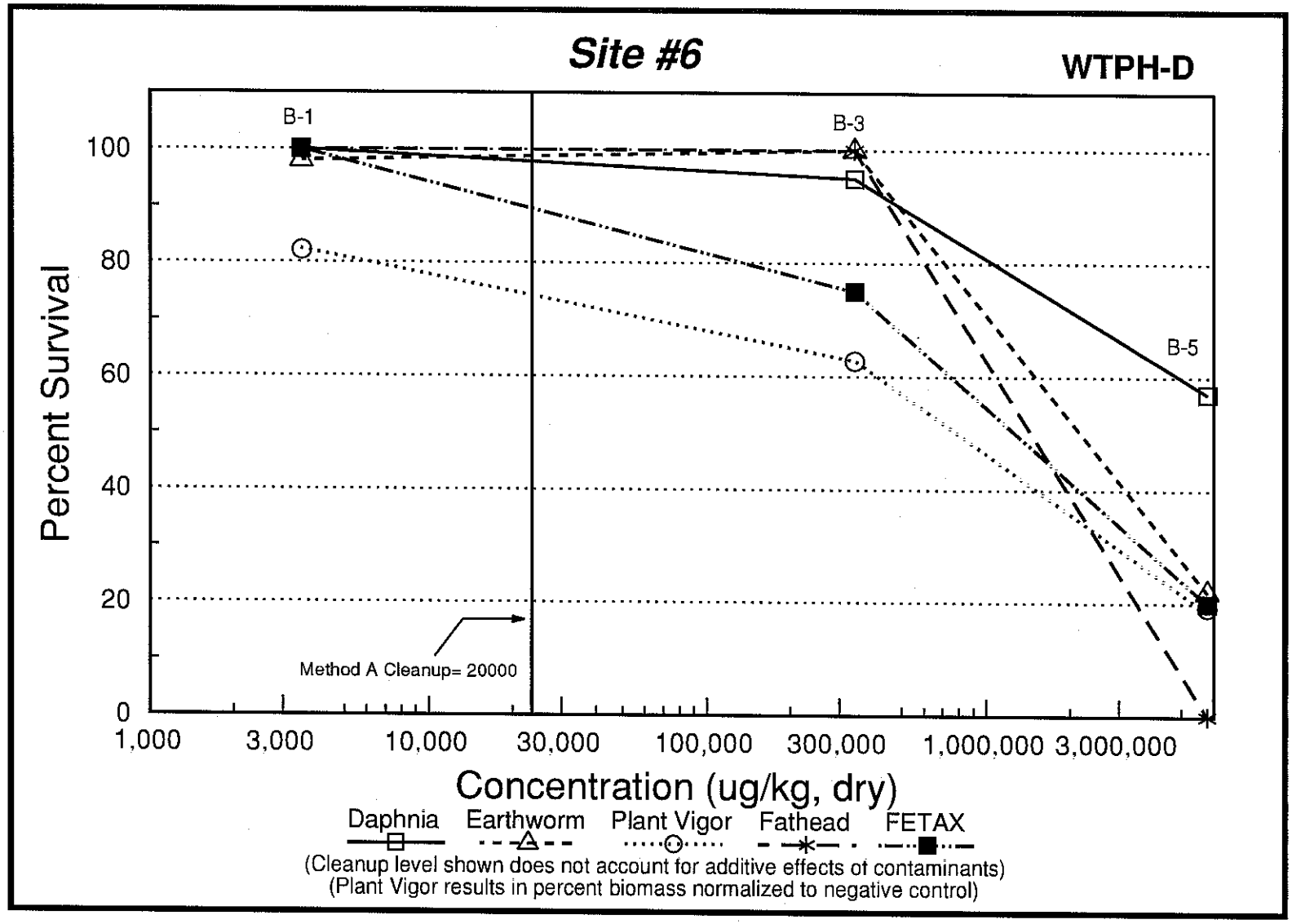

Figure 8:Response of bioassay suite to primary contaminants in soils at Site #6.

- Comparing the bioassay responses to the MTCA residential soil cleanup levels suggests that the established standards for the protection of human health might not adequately protect some species in the ecological community.

- Problems encountered in conducting the five tier-one screening levels bioassays included: poor seed germination (<90%) in the negative control for the first batch of Plant Vigor samples; procedures for maintaining soil moisture conditions in both the Earthworm and Plant Vigor tests are inadequate, and a limited amount of sample was available in the first batch of samples for the Earthworm and Plant Vigor tests.

RECOMMENDATIONS

Based on the results of the Soil Bioassay Pilot Study the following recommendations are made:

- Based on a review of the results from this study (pH of sample extracts) and established procedures for conducting FETAX, distilled water should be used as an extractant for preparing the sample eluates. FETAX solution should then be added to the eluate prior to addition of Xenopus embyos.

- Water holding capacity and testing procedures in the Earthworm and Plant Vigor Protocols should be modified to better related field soil moisture and laboratory testing conditions. The goal of these modifications would be to eliminate the need to rehydrate the samples during the course of the study.

- Collect samples for biological/chemical testing on petroleum contaminated (especially gasoline) soils during the time of excavation. This will minimize the loss of volatile compounds.

- Sieving of samples should be avoided if possible. Especially if the contaminants of concern have the potential to volatilize. Sieving should be limited to soils with a high percentage of gravel. A 1/4" mesh screen is recommended for sieving when needed.

- A separate sample should be collected for the soil characterization work specified in the bioassay protocols. It is recommended that to analyze three replicates for each sample, at a minimum the following volumes should be submitted to the laboratory: Soil Characterization- 8oz; Bioassay- *Daphnia* 16oz, Earthworm 16oz, Plant Vigor 48oz, Fathead Minnow 16oz , and FETAX 8oz.

- Chemical characterization work should be conducted in conjunction with the bioassays.

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APPENDIX

Appendix A

Sampling Gear and Soil Descriptions

Table A1: Description of sampling gear and soil types for the Soil Bioassay Pilot Study.

I. Western Washington (February 8–10, 1993)

Station	Sampling Gear	Screen (mm)	Soil Description
Site #1- Metals			
WD1	HA	2	brown sandy loam
WD2	HA	2	gravelly sand
WD3	HA	2	light gray disintegrating gravel
Site #2- Creosote			
CP1	HA	6.7	brown sandy gravel w/some shell fragments
CP2	Backhole	6.7	dark brown sandy gravel
CP3	HA	6.7	oily black sandy gravel w/shell fragments
Site #3- Petroleum Products			
JC1	Spoon	6.7	clayey silt w/some gravel
JC2	Backhole	6.7	sandy clay w/sparse gravel
JC3	Backhole	None	sandy blue clay- moist

II. Eastern Washington (March 1–3, 1993)

Station	Sampling Gear	Screen (mm)	Soil Description
Site #4- Metals			
AM1	HA	None	light brown silty sand
AM2	HA	None	fine silty sand w/yellow layering
AM3	HA	None	fine silty sand w/yellow layering
Site #5- Pesticides			
RA1	HA	None	brown silty sand
RA2	HA	None	brown silty sand
RA3	HA	None	brown silty sand
Site #6			
B1	HA	None	silty clay w/some organic debris- moist
B2	HA	None	sandy fill w/limited gravel
B3	HA	None	sandy silt
B4	HA	6.7	sandy gravel fill
B5	HA	None	saturated clay w/diesel odor

HA= Hand operated stainless steel bucket auger

Appendix B

Case Narratives for Chemical Analyses

STATE OF WASHINGTON
DEPARTMENT OF ECOLOGY
MANCHESTER ENVIRONMENTAL LABORATORY
7411 Beach Drive East • Port Orchard, Washington 98366-8204 • (206) 871-8860 • SCAN 871-8860

April 21, 1993

TO: Project Officer

FROM: David A Thomson DAT

SUBJECT: Quality Assurance memo for the Soil Bioassay Project

SAMPLE RECEIPT

The samples from the Soil Bioassay project were received by the Manchester Laboratory on February 11, 1993 in good condition. The analyses for these samples were subsequently contracted to Analytical Resources Inc. The samples were run by the following methods:

Total Organic Carbon PSEP
Sulfide PSEP
Ammonia ~~EPA Method 350.1~~ Plumb, 1981 ICL Extraction

HOLDING TIMES

All analyses were performed within the USEPA method holding times.

INSTRUMENT CALIBRATION

Instrument calibration was performed before each analytical run and checked by initial calibration verification standards and blanks.

PROCEDURAL BLANKS

Delete DN 6/8/93 ~~The procedural blanks associated with these samples showed no analytically significant levels of analytes. It should be noted that all Ammonia results except samples 078210, 078211 and 078222 are less that 10 times the detection limit and should be qualified as P.~~

SPIKED SAMPLE ANALYSIS

Spike and duplicate spike sample analyses were performed on sample number 078205. All spike recoveries were within limits of +/- 20%.

PRECISION DATA

The results of sample 078205 run in duplicate were used to evaluate precision on this sample set. The Relative Percent Difference (RPD) for all analytes was within the +/- 10% window for duplicate analysis.

LABORATORY CONTROL SAMPLE (LCS) ANALYSES

LCS analyses were within the windows established for each parameter.

SUMMARY

The data generated by the analysis of these samples can be used noting the data qualifications discussed in this memo.

Please call David A Thomson at SCAN 871-8822 to further discuss this project.

STATE OF WASHINGTON
DEPARTMENT OF ECOLOGY
MANCHESTER ENVIRONMENTAL LABORATORY
7411 Beach Drive East • Port Orchard, Washington 98366-8204 • (206) 871-8860 • SCAN 871-8860

April 28, 1993

TO: Project Officer

FROM: David A Thomson

SUBJECT: Quality Assurance memo for the Soil Bioassay Project

SAMPLE RECEIPT

The samples from the Soil Bioassay project were received by the Manchester Laboratory on March 5, 1993 in good condition. The analyses for these samples were subsequently contracted to Analytical Resources Inc. The samples were run by the following methods:

Total Organic Carbon PSEP
Sulfide PSEP
Ammonia ~~EPA Method 350.1~~ Plumb, 1981 KCL Extraction

HOLDING TIMES

All analyses were performed within the USEPA method holding times.

INSTRUMENT CALIBRATION

Instrument calibration was performed before each analytical run and checked by initial calibration verification standards and blanks.

PROCEDURAL BLANKS

Delete DN 6/9/93 ~~The procedural blanks associated with these samples showed no analytically significant levels of analytes. It should be noted that all Ammonia results except samples 108235, 108236, 108240, and 108241 are less that 10 times the detection limit and should be qualified as P.~~

SPIKED SAMPLE ANALYSIS

Spike and duplicate spike sample analyses were performed. All spike recoveries were within limits of +/- 20%.

PRECISION DATA

The results of samples run in duplicate were used to evaluate precision on this sample set. The Relative Percent Difference (RPD) for all analytes was within the +/- 15% window for duplicate analysis.

LABORATORY CONTROL SAMPLE (LCS) ANALYSES

LCS analyses were within the windows established for each parameter.

SUMMARY

The data generated by the analysis of these samples can be used noting the data qualifications discussed in this memo.

Please call David A Thomson at SCAN 871-8822 to further discuss this project.

State of Washington Department of Ecology
Manchester Environmental Laboratory
7411 Beach Dr East Port Orchard WA 98366

Data Review
March 14, 1993

Project: **Soil Bioassay** KF

Samples: 078205 through 078255

Laboratory: Soil Technology J-326

By: Karin Feddersen

Case Summary

The review is for sediment grain size using Puget Sound Estuary Program (P.S.E.P.) protocol.

These samples were received at the Manchester Environmental Laboratory on March 5, 1993. They were transported to Soil Technology on March 8, 1993 for analysis.

These analyses were reviewed for qualitative and quantitative accuracy, validity, and usefulness. The results are acceptable for use as reported.

STATE OF WASHINGTON
DEPARTMENT OF ECOLOGY
MANCHESTER ENVIRONMENTAL LABORATORY
7411 Beach Drive East • Port Orchard, Washington 98366-8204 • (206) 895-4737 • SCAN 744-4737

April 2, 1993

TO: Dale Norton

FROM: Bill Kammin, Environmental_Lab_Director BK

SUBJECT: Metals Quality Assurance memo for the Soil Bioassay Project (Batch 1)

SAMPLE INFORMATION

These samples from the Soil Bioassay project were received by the Manchester Laboratory on 2/11/93 in good condition.

HOLDING TIMES

All analyses were performed within the USEPA Contract Laboratory Program (CLP) holding times for metals analysis (28 days for mercury, 180 days for all other metals).

INSTRUMENT CALIBRATION

Instrument calibration was performed before each analytical run and checked by initial calibration verification standards and blanks. Continuing calibration standards and blanks were analyzed at a frequency of 10% during the run and again at the end of the analytical run. All initial and continuing calibration verification standards were within the relevant USEPA (CLP) control limits. AA calibration gave a correlation coefficient (r) of 0.995 or greater, also meeting CLP calibration requirements.

PROCEDURAL BLANKS

The procedural blanks associated with these samples showed no analytically significant levels of analytes, with the following exception: zinc. Samples with zinc levels less than 10 times blank levels are qualified with B.

SPIKED SAMPLE ANALYSES

Spike and duplicate spike sample analyses were performed on this data set. All spike recoveries were within the CLP acceptance limits of +/- 25%, with the following exception: antimony. Antimony results are qualified with J, denoting estimated values because of low spike recoveries and low LCS recovery.

PRECISION DATA

The results of the spike and duplicate spike samples were used to evaluate precision on this sample set. The Relative Percent Difference (RPD) for all analytes was within the 20% CLP acceptance window for duplicate analysis.

LABORATORY CONTROL SAMPLE (LCS) ANALYSES

LCS analyses were within the windows established for each parameter, with the following exception: antimony. See the attached LCS worksheet for further details.

SERIAL DILUTION ANALYSES

Serial dilution is used in ICP analyses to examine sample results for potential interferences. The serial dilution results for this sample set met CLP specifications.

SUMMARY

Current EPA regulatory methods for the preparation of solid matrices do not recover antimony from complex matrices. These poor recoveries are well known to EPA and other regulatory agencies. For this project, antimony was not detected, and all results are qualified as UJ.

The data generated by the analysis of these samples can be used noting the data qualifications discussed in this memo.

Please call Bill Kammin at SCAN 744-4737 to further discuss this project.

WRK:wrk

STATE OF WASHINGTON
DEPARTMENT OF ECOLOGY
MANCHESTER ENVIRONMENTAL LABORATORY
7411 Beach Drive East • Port Orchard, Washington 98366-8204 • (206) 895-4737 • SCAN 744-4737

May 4, 1993

TO: Dale Norton

FROM: Bill Kammin, Environmental_Lab_Director

SUBJECT: Metals Quality Assurance memo for the Soil Bioassay Project

SAMPLE INFORMATION

These samples from the Soil Bioassay project were received by the Manchester Laboratory on 3/5/93 in good condition.

HOLDING TIMES

All analyses were performed within the USEPA Contract Laboratory Program (CLP) holding times for metals analysis (28 days for mercury, 180 days for all other metals).

INSTRUMENT CALIBRATION

Instrument calibration was performed before each analytical run and checked by initial calibration verification standards and blanks. Continuing calibration standards and blanks were analyzed at a frequency of 10% during the run and again at the end of the analytical run. All initial and continuing calibration verification standards were within the relevant USEPA (CLP) control limits. AA calibration gave a correlation coefficient (r) of 0.995 or greater, also meeting CLP calibration requirements. For arsenic, chromium and nickel, the closing interference check solution was slightly outside of acceptance windows. Data for these elements is qualified with J, denoting estimated values.

PROCEDURAL BLANKS

The procedural blanks associated with these samples showed no analytically significant levels of analytes.

SPIKED SAMPLE ANALYSES

Spike and duplicate spike sample analyses were performed on this data set. All spike recoveries were within the CLP acceptance limits of +/- 25%, with the following

exception: antimony. Antimony results are qualified with J, denoting estimated values.

PRECISION DATA

The results of the spike and duplicate spike samples were used to evaluate precision on this sample set. The Relative Percent Difference (RPD) for all analytes was within the 20% CLP acceptance window for duplicate analysis.

LABORATORY CONTROL SAMPLE (LCS) ANALYSES

LCS analyses were within the windows established for each parameter, with the following exception: antimony.

SERIAL DILUTION ANALYSES

Serial dilution is used in ICP analyses to examine sample results for potential interferences. The serial dilution results for this sample set met CLP specifications.

SUMMARY

The data generated by the analysis of these samples can be used noting the data qualifications discussed in this memo.

Please call Bill Kammin at SCAN 206-871-8801 to further discuss this project.

WRK:wrk

State of Washington Department of Ecology
Manchester Environmental Laboratory
7411 Beach Dr. East Port Orchard WA. 98366

April 16, 1993

Project: **Soil Bioassay**

Samples: 108242 through 108248

Laboratory: Analytical Resources Inc. D169

By: Karin Feddersen KF

Case Summary

These samples were received at the Manchester Environmental Laboratory on March 5, 1993, and transported to Analytical Resources, Inc. on March 8, 1993 for Pesticides/PCB's, Organophosphorous Pesticides, and Herbicides analysis.

These analyses were reviewed for qualitative and quantitative accuracy, validity, and usefulness.

There is no need to assimilate the "dilution factor" or "sample wt/vol" into the final values reported; these calculations have already been figured into the reported values.

DATA QUALIFIER DEFINITIONS

U - The analyte was not detected at or above the reported result.

UJ - The analyte was not detected at or above the reported estimated result.

J - The associated numerical result is an estimated quantity.

Pesticides/PCB's

Holding Times:

As mentioned in ARI's narrative, these samples were re-extracted four days past the SW-846 recommended holding times. Since the samples were stored in the proper container at the proper temperature, and the reanalysis exhibited comparable results, extraction four days beyond the recommended holding times is not considered to have significantly affected the results.

Method Blank:

No target analytes were detected in the method blank.

Initial Calibration:

The initial calibration % Relative Standard Deviations were within the maximum of 20%.

Continuing Calibrations:

The percent deviations between the initial and continuing calibration standards were within the maximum of 15%.

Matrix Spikes (MS/MSD):

Matrix spike recovery and precision data are reasonable, acceptable, and within advisory QC limits.

Surrogates:

Surrogate recoveries for these samples, the matrix spikes, and the associated method blank are reasonable, acceptable, and within advisory QC limits.

Sample Data:

This data is acceptable for use without the need for additional data qualifiers.
The "X" qualifier is used by ARI to indicate that the associated result was derived from a response that exceeded the calibration range, and a dilution analysis was required. This "X" has been replaced by a "J" qualifier to indicate an estimated value. Use the dilution analyses for samples 108245 and 108246 for the Aldrin, Endosulfan I and II, Dieldrin, and 4,4'-DDT results; for all the other analytes use the undiluted analyses.

Organophosphorous Pesticides

Holding Times:

These samples were extracted and analyzed within the SW-846 recommended holding times.

Method Blank:

No target analytes were detected in the method blank.

Initial Calibration:

The initial calibration % Relative Standard Deviations were within the maximum of 20%.

Continuing Calibrations:

The percent deviations between the initial and continuing calibration standards were within the maximum of 15%.

Matrix Spikes (MS/MSD):

Matrix spike recovery and precision data are reasonable, acceptable, and within advisory QC limits.

Surrogates:

Surrogate recoveries for these samples, the matrix spikes, and the associated method blank are reasonable, acceptable, and within advisory QC limits.

Sample Data:

This data is acceptable for use without the need for additional data qualifiers.

Herbicides

Holding Times:

These samples were extracted and analyzed within the SW-846 recommended holding times

Method Blank:

No analytes were detected in the method blank.

Initial Calibration:

The initial calibration % Relative Standard Deviations were within the maximum of 20%.

Continuing Calibrations:

The percent deviations between the initial and continuing calibration standards were within the maximum of 15%.

Surrogates:

Surrogate recoveries for these samples and the associated method blank are reasonable, acceptable and within QC limits.

Sample Data:

This data is acceptable for use with the additional data qualifiers where appropriate.

Round 2

STATE OF WASHINGTON
DEPARTMENT OF ECOLOGY
MANCHESTER ENVIRONMENTAL LABORATORY
7411 Beach Drive East • Port Orchard, Washington 98366-8204 • (206) 895-4737 • SCAN 744-4737

March 26, 1993

TO: Dale Norton

FROM: Bob Carrell

SUBJECT: Soil Bioassay Study (Bingo)

I felt that it may be of interest to you that samples 93108235, 36 and 37 all contained extremely weathered diesel oil where all of the major straight chain hydrocarbons had been lost. This is probably due to microbial activity. Samples 93108240 and 41 had diesel oil which, although weathered, still contained the major straight chain hydrocarbons, but at smaller quantities than normal. It would appear that, given that these samples all were subjected to the same environmental conditions, the diesel in samples 93108240 and 41 is a more recent addition to the environment and 93108235-37's diesel.

Further, the gasoline in samples 9310833 and 34 was very weathered and has lost the entire BTEX range hydrocarbons.

BC:dh

cc: Bill Kammin

State of Washington Department of Ecology
Manchester Environmental Laboratory
7411 Beach Dr. East Port Orchard WA. 98366

Data Review
March 15, 1993

Project: **Soil Bioassay**

Samples: 078212, 078213, 078214, 078215, 078216, 078217, 078218

Laboratory: Sound Analytical Services, Inc 30184

By: Karin Feddersen

Case Summary

These samples were received at the Manchester Environmental Laboratory on February 1, 1993, and transported to Sound Analytical Services, Inc. on February 3, 1993 for BTEX and WTPH-G analyses.

These analyses were reviewed for qualitative and quantitative accuracy, validity, and usefulness

There is no need to assimilate the "dilution factor" or "sample wt/vol" into the final values reported; these calculations have already been figured into the reported values.

To keep a consistent report format, the qualifier "U" has been added to non-detected compounds.

DATA QUALIFIER DEFINITIONS

U - The analyte was not detected at or above the reported result.

UJ - The analyte was not detected at or above the reported estimated result.

J - The associated numerical result is an estimated quantity.

BTEX

Holding Times:

These samples were analyzed within the SW-846 recommended holding time.

Method Blank:

No target analytes were detected in either method blank.

Initial Calibration:

The eight-point initial calibration for samples 078213 through 078218 exhibited a poor response for the first four calibration points. The values for these samples have been recalculated. A calibration curve based on the higher four concentration standards was used. The % Relative Standard Deviations for this curve were within the maximum of 20%. Positive results for all analytes that fell below the lowest acceptable calibration point have been qualified with a "J" and non-detected results have been qualified with a "UJ" in the corresponding samples.

Continuing Calibration:

The average relative response factors for all target analytes were above the minimums, and the percent deviations between the initial and continuing calibration standards were within the maximum of 15%.

Surrogates:

Surrogate recoveries for these samples and the associated method blanks are reasonable, acceptable, and within QC limits, with several exceptions. The surrogate recoveries for the dilution analyses of samples 078217 and 078218 were most likely out of QC limits because the dilution required to bring some of the detected analytes into the range of the calibration curve resulted in surrogate quantities that fell well below the calibration curve. Hence the recoveries were estimated from an extrapolation of the calibration curve. Neither of these outliers indicates an out of control analysis. Target analytes were detected in samples 078217 and 078218 above the regulatory levels allowed for soil, and therefore re-analysis or qualification of the results is not warranted.

Sample results:

This data is acceptable for use as amended.

WTPH-G

Holding Times:

These samples were analyzed within the maximum suggested holding time.

Method Blank:

No target compounds were detected in either method blank.

Initial Calibration:

The initial calibration met the minimum response criteria for the average relative responses. The % Relative Standard Deviations were within the maximum of 20%.

Continuing Calibration:

The average relative response factors for the target analytes were above the minimums, and the percent deviations between the initial and continuing calibration standards were within the maximum of 15%.

Surrogates:

Surrogate recoveries for this sample and the associated method blanks are reasonable, acceptable, and within QC limits, with several exceptions. The surrogate recoveries for the dilution analyses of samples 078217 and 078218 were most below QC limits because the dilution required to bring some of the detected analytes into the range of the calibration curve resulted in surrogate quantities that fell well below the calibration curve. Hence the recoveries were estimated from an extrapolation of the calibration curve. Neither of these outliers indicates an out of control analysis. Low surrogate recovery is an indication that the results may be biased low. However, since target analytes were detected in samples 078217 and 078218 above the regulatory levels, re-analysis is not deemed necessary.

Sample results:

This data is acceptable for use as amended.

MANCHESTER ENVIRONMENTAL LABORATORY

7411 Beach Drive E , Port Orchard Washington 98366

CASE NARRATIVE

April 1, 1993

Subject: Soil Bioassay Study

Samples: 93 - 108230 to -108234, -108238 and -108239

Case No. DOE-643Y

Officer: Dale Norton

By: Dickey D. Huntamer
Organics Analysis Unit

BETX ANALYSIS

ANALYTICAL METHODS:

The samples were analyzed by EPA Method SW-846 - 8020. Normal QA/QC procedures were performed on the samples.

HOLDING TIMES:

The samples were analyzed within the recommended holding times.

BLANKS:

No target compounds were detected in the laboratory blank. The EPA five times rule was applied to all target compounds which were found in the blank. Compounds that were found in the sample and in the blank were considered real and not the result of contamination if the levels in the sample are greater than or equal to five times the amount of compounds in the associated method blank.

SURROGATES:

Surrogate recoveries were within acceptable limits.

MATRIX SPIKE AND MATRIX SPIKE DUPLICATE:

A matrix spike and spike duplicate was analyzed using sample -108230. Recoveries ranged from 105% to 118% and Relative Percent Differences (RPD) ranged from 6.2% to 10.4%. Recoveries and precision data were within acceptable limits.

ANALYTICAL COMMENTS:

No problems were encountered in the analysis of these samples. The data is acceptable to use without additional qualifiers.

DATA QUALIFIER CODES:

U	-	The analyte was not detected at or above the reported value
J	-	The analyte was positively identified. The associated numerical value is an estimate.
UJ	-	The analyte was not detected at or above the reported estimated result.
REJ	-	The data are unusable for all purposes.
EXP	-	The result is equal to the number before EXP times 10 to the power of the number after EXP. As an example 3EXP6 equals 3×10^6.
NAF	-	Not analyzed for.
N	-	For organic analytes there is evidence the analyte is present in this sample.
NJ	-	There is evidence that the analyte is present. The associated numerical result is an estimate.
E	-	This qualifier is used when the concentration of the associated value exceeds the known calibration range.
*	-	The analyte was present in the sample. (Visual Aid to locate detected compound on report sheet.)

MANCHESTER ENVIRONMENTAL LABORATORY

7411 Beach Drive E , Port Orchard Washington 98366

CASE NARRATIVE

April 12, 1993

Subject: Soil Bioassay

Samples: 93 - 078212 to -078224

Case No. DOE-622Y

Officer: Dale Norton

By: Dickey D. Huntamer
Organics Analysis Unit

SEMIVOLATILE ORGANICS

ANALYTICAL METHODS:

The semivolatile water samples were extracted with methylene chloride following the Manchester modification of the EPA CLP and SW 846 8270 procedure with capillary GC/MS analysis of the sample extracts. Normal QA/QC procedures were performed with the analyses.

HOLDING TIMES:

All sample extraction and analysis holding times were met.

BLANKS:

Some target analytes were detected in the laboratory blanks. The EPA 5 times rule was applied to all target compounds which were found in the blank. Compounds that were found in the sample and in the blank were considered real and not the result of contamination if the levels in the sample are five times or greater than compounds in the method blank.

SURROGATES:

All surrogate recoveries were within acceptable limits.

MATRIX SPIKE AND MATRIX SPIKE :

Matrix spike recoveries were within acceptable limits for most of the target compounds. Three compounds, 2,4-dimethylphenol, 4-chloroaniline, and 3-nitroaniline in sample -078216 had the "J" qualifier added because their recoveries were outside acceptable limits.

ANALYTICAL COMMENTS:

No significant problems were encountered in he analysis. The data is acceptable for use as qualified.

DATA QUALIFIER CODES:

U	-	The analyte was not detected at or above the reported value.
J	-	The analyte was positively identified. The associated numerical value is an estimate.
UJ	-	The analyte was not detected at or above the reported estimated result.
REJ	-	The data are unusable for all purposes.
EXP	-	The result is equal to the number before EXP times 10 to the power of the number after EXP. As an example 3EXP6 equals 3×10^6.
NAF	-	Not analyzed for.
N	-	For organic analytes there is evidence the analyte is present in this sample.
NJ	-	There is evidence that the analyte is present. The associated numerical result is an estimate.
E	-	This qualifier is used when the concentration of the associated value exceeds the known calibration range.
*	-	The analyte was present in the sample. (Visual Aid to locate detected compound on report sheet.)

MANCHESTER ENVIRONMENTAL LABORATORY

7411 Beach Drive E , Port Orchard Washington 98366

CASE NARRATIVE

April 27, 1993

Subject: Soil Bioassay II

Samples: 93 - 108030 to -108041

Case No. DOE-643Y

Officer: Dale Norton

By: Dickey D Huntamer
Organics Analysis Unit

SEMIVOLATILE ORGANICS

ANALYTICAL METHODS:

The semivolatile water samples were extracted with methylene chloride following the Manchester modification of the EPA CLP and SW 846 8270 procedure with capillary GC/MS analysis of the sample extracts. Normal QA/QC procedures were performed with the analyses.

HOLDING TIMES:

All sample extraction and analysis holding times were met.

BLANKS:

Some target analytes were detected in the laboratory blanks. The EPA 5 times rule was applied to all target compounds which were found in the blank. Compounds that were found in the sample and in the blank were considered real and not the result of contamination if the levels in the sample are five times or greater than compounds in the method blank.

SURROGATES:

All surrogate recoveries were within acceptable limits, except for 93-108035 where 2-florophenol, d4-2-chlorophenol, 1,2-dichlorobenzene and d5-nitrobenzene all had low recoveries, 6-14%. The results for the compounds associated with these surrogates were qualified using the "J" flag.

MATRIX SPIKE AND MATRIX SPIKE :

Matrix spike recoveries were within acceptable limits for most of the target compounds. Several compounds, 2,4-dimethylphenol, 4-nitroaniline, 3-nitroaniline and benzo(g,h,i)perylene were qualified in sample 93-108030 with a "J" because their recoveries were outside acceptable limits. One compound, 4-chloroaniline was given the "REJ" flag due to low recoveries.

ANALYTICAL COMMENTS:

No significant problems were encountered in he analysis. The data is acceptable for use as qualified.

DATA QUALIFIER CODES:

U	-	The analyte was not detected at or above the reported value.
J	-	The analyte was positively identified. The associated numerical value is an estimate.
UJ	-	The analyte was not detected at or above the reported estimated result.
REJ	-	The data are unusable for all purposes.
EXP	-	The result is equal to the number before EXP times 10 to the power of the number after EXP. As an example 3EXP6 equals 3×10^6.
NAF	-	Not analyzed for.
N	-	For organic analytes there is evidence the analyte is present in this sample.
NJ	-	There is evidence that the analyte is present. The associated numerical result is an estimate.
E	-	This qualifier is used when the concentration of the associated value exceeds the known calibration range.
*	-	The analyte was present in the sample. (Visual Aid to locate detected compound on report sheet.)

Appendix C

Bioassay Results

Case Narratives

STATE OF WASHINGTON
DEPARTMENT OF ECOLOGY
MANCHESTER ENVIRONMENTAL LABORATORY
7411 Beach Drive East • Port Orchard, Washington 98366-8204 • (206) 871-8860 • SCAN 871-8860

May 27, 1993

TO: Dale Norton
Environmental Investigations

FROM: Margaret Stinson, Supervisor
Scott Noble, Analyst
Cherlyn Milne, Analyst
Manchester Aquatic Toxicology Unit

SUBJECT: Soil Bioassay Study
Results of Toxicity Testing

INTRODUCTION

The Department of Ecology has developed draft protocols to evaluate soils from hazardous waste sites in the State of Washington. Use of such testing procedures would facilitate cleanup efforts as a measure of their success. This study was designed to test the draft protocols, evaluating the relative sensitivity of the test organisms to a variety of toxicants, and identifying ways to improve the draft protocol documents.

A total of forty samples were collected from six sites, three in Western Washington, three in Eastern Washington. All samples were tested using *Daphnia magna*, Plant Vigor, and Earthworm protocols. Half of the samples from each site were tested using FETAX and Fathead Minnow. Plant Vigor, Earthworm, and FETAX tests were conducted by contractors with the equipment and experience required for those tests.

The *Daphnia magna* and Fathead Minnow tests were conducted at Manchester Laboratory. Test methods, results, and raw data for those tests are presented in this report.

SAMPLE DESCRIPTION

The samples were collected over the period from February 8-10, for Western Washington Sites, and March 1-3, for Eastern Washington Sites. Samples were held

on ice until delivered to Manchester Laboratory. Individual sample descriptions are in Appendix I.

METHODS

Testing was conducted following the methods described in the draft documents Latier and Landis (1992) *Daphnia Survival Toxicity Test,* and (1992a) *Fathead Minnow Toxicity Test,* prepared for Washington State Department of Ecology. Prior to testing a 125 gm aliquot of each sample was placed in a 250 ml Pyrex beaker, dried for 24 hours at 105°C, and weighed to obtain percent moisture. Soil moisture data are presented in Appendix II.

A single extract of each sample was prepared for both *Daphnia magna* and Fathead Minnow testing. Samples were extracted into reconstituted moderately hard laboratory water (deionized water adjusted to 80-90 mg/L $CaCO_3$ and aerated). Extraction was by placing an aliquot in dilution water equal to four times the calculated dry weight of the aliquot in a two liter Nalgene bottle; the mixture was placed on a rotary extractor and shaken at 30 rpm for 48 hours in darkness at 22±2°C. The mixture was allowed to settle overnight at 4° C before decanting. The decanted eluate was centrifuged until clear, normally about 20 to 30 minutes, using a Sorvall RC3C refrigerated (4C°) centrifuge at 4200 rpm.

The overlying liquid was then decanted and, if necessary, the pH was adjusted to between 7.0 and 8.6, as required by the *Daphnia* test protocol. Several samples had pH measurements lower than 7.0; these were adjusted using sodium hydroxide solution (50 gm/liter). Table 1 describes pH adjustments made to test extracts. The solution was also aerated if the measured dissolved oxygen was below 60% saturation either before or during testing. Table 2 describes aeration of test extracts. The test solutions were allowed to equilibrate at 20°C in preparation for testing.

A deviation from the test protocol occurred during extraction of samples 10-8244 through 10-8255. Samples were not removed from the rotary extractor at 48-hours, but continued to shake overnight, due to analyst error. The samples were allowed to settle for one hour, rather than overnight, before centrifugation of the supernatants. The total contact time for the extraction was the same as required by the protocol. It is unlikely that this change had a significant effect upon the outcome of testing. If an effect was to result from this deviation, it would likely be to show additional toxicity; the higher temperature and tumbling action during the overnight period might extract additional toxicants.

Test procedures for each of the organisms are described below.

Table 1. Sample extracts requiring pH adjustment before testing, including initial and adjusted pH, and the volume of sodium hydroxide solution (50 gm/L) required to neutralize the eluate.

Site Description	Sample Number	Initial pH	Adjusted pH	Volume NaOH (ml)
WD-1A	07-8205	6.39	7.56	0.2
WD-1B	07-8206	6.34	7.43	0.4
WD-2A	07-8207	5.09	7.72	0.24
WD-2B	07-8208	5.10	7.45	0.32
WD-3A	07-8210	5.41	7.90	0.68
WD-3B	07-8211	5.36	7.36	0.48
~~L-1A~~ JC-1A	07-8212	6.64	7.52	0.2
~~L-1B~~ JC-1B	07-8213	6.61	7.72	0.24
~~L-2A~~ JC-2A	07-8214	5.93	7.68	0.36
~~L-2B~~ JC-2B	07-8215	5.99	7.72	0.52
~~L-3A~~ JC-3A	07-8217	5.54	7.80	1.0
~~L-3B~~ JC-3B	07-8218	5.62	7.69	1.08
B-1A	10-8230	6.15	7.91	0.56
B-2A	10-8231	6.12	7.61	0.60
B-3A	10-8235	6.27	7.79	0.76
B-3B	10-8236	6.25	7.70	0.60
AM-2A	10-8251	2.17	7.13	43*
AM-2B	10-8252	2.14	7.14	45*
AM-3A	10-8254	3.57	7.66	7.85**
AM-3B	10-8255	3.62	7.21	8.10**

*Neutralization produced color changes from yellow to orange to khaki-colored floc which settled quickly

**Neutralization produced a pale blue-colored floc which settled quickly

Table 2. Sample extracts requiring aeration before testing when measured dissolved oxygen (DO) concentrations were less than 60% saturation, including DO concentrations before and after aeration. Aeration duration was approximately one hour unless otherwise noted.

Site Description	Sample Number	Initial DO (mg/L)	Adjusted DO (mg/L)
~~L-3B~~ JC-3B	07-8218	5.2	8.8
CP-3A	07-8223	4.0	8.1
CP-3B	07-8224	4.1	7.5
B-3A	10-8235	4.6	8.4
B-3B	10-8236	4.5	8.4
B-5A	10-8240	3.3	7.3
B-5B	10-8241	2.3	8.1
AM-2A	10-8251	<1*	7.0
AM-2B	10-8252	<1*	6.3

*Aeration for 1 hour increased DO to 2-3 mg/L; the test solutions were aerated overnight to achieve DO appropriate for testing.

Daphnia magna

Test organisms were obtained from cultures maintained at Manchester Laboratory. Organisms were conditioned for four weeks prior to initiation of testing. From each extract, 200 ml of test solution was placed in each of three 250 ml Pyrex beakers. Control beakers were prepared in the same way using reconstituted moderately hard laboratory water. Ten neonate (<24-hours old) *Daphnia magna* were randomly distributed to each of the beakers which were placed in a 20±1°C incubator with a 16/8 light/dark photoperiod for the duration of the test. The test vessels were observed at 24 hours, counting and removing mortalities.

Dissolved oxygen and pH were measured at 0, 24, and 48 hours. Aliquots were taken from one replicate of each sample eluate to analyze for hardness, alkalinity, and conductivity at test initiation and termination.

Cadmium chloride (EMSL/EPA, Cincinnati) was used as a reference toxicant. The suggested range of concentrations (1.2 to 19.2 ug/L) was used during the first set of testing, however the LC50 was not bracketed. The range was expanded (1 to 40 ug/L) for the second set of tests, successfully estimating the LC50.

Because extraction was limited by equipment availability, extraction and testing was done in two batches for each of the two sets of samples received. For the first set of samples, testing was initiated on February 19, and February 21. For the second set, testing was initiated on March 11, and March 13. The two samples requiring overnight aeration, 10-8251 and 10-8252, were tested beginning March 14. Reference toxicant tests were initiated on February 18, and on March 10. Eluate and reference toxicant tests were terminated after 48 hours by examining each replicate and recording numbers of survivors.

Survival data were tested for statistical significance relative to control responses using Toxstat 3.3 (University of Wyoming, 1991). The LC50s for the reference toxicant were estimated by the Trimmed Spearman-Karber Method using software provided by EPA (EMSL, Cincinnati), and checked using the graphical method.

Fathead Minnow

Test organisms were obtained from Aquatic Resources, a commercial supplier. Two-day-old fish were received 24-hours before initiation of testing. Fish were equilibrated at test temperature in a 50/50 mixture of dechlorinated Manchester City water and moderately hard reconstituted water and fed newly hatched *Artemia* prior to testing.

From each extract, 100 ml of test solution was placed in each of three 150 ml Pyrex beakers. Control beakers were also prepared using reconstituted moderately hard water. Ten fathead minnow larvae were randomly distributed to each of the beakers;

The test beakers were placed in an incubator at 20±1°C, with a 16/8 light/dark photoperiod for the duration of the test. The test vessels were observed at 24 hours, counting and removing mortalities.

Dissolved oxygen and pH were measured at 0, 24, and 48 hours. Aliquots were taken from one replicate of each sample eluate to analyze for hardness, alkalinity, and conductivity at test initiation and termination.

Cadmium chloride (EMSL/EPA, Cincinnati) was used as a reference toxicant. The suggested range of concentrations (9.9 to 144 ug/L cadmium) was used during the first set of testing, however the LC50 was not bracketed. The range was expanded (10 to 810 ug/L cadmium) for the second set of tests, successfully bracketing the LC50.

As with *Daphnia magna*, extraction and testing was done in two batches for each of the two sets of samples received. For the first set of samples, testing was initiated on February 19, and February 21. For the second set, testing was initiated on March 11, and March 13. The two samples requiring overnight aeration, 10-8251 and 10-8252, were tested beginning March 14; as a result, the test organisms were six-days-old at test initiation. Reference toxicant tests were initiated on February 19, and on March 11. Eluate and reference toxicant tests were terminated after 48 hours by examining each replicate and recording numbers of survivors.

Survival data were tested for statistical significance relative to control responses using Toxstat 3.3 (University of Wyoming, 1991). The LC50s for the reference toxicant were estimated by the Trimmed Spearman-Karber Method using software provided by EPA (EMSL, Cincinnati), and checked using the graphical method.

RESULTS

Toxicity test results, including extract chemistry analyses, are in Appendix III. Test results are summarized in Tables 3 and 4.

The two test organisms were consistent in their non-toxic responses to samples; all but the highest numbered sample from each site (except RA-3) had 90-100% survival in tests of both organisms. They did not always respond consistently to toxicity, however. Both organisms responded negatively to samples L-3, CP-3, and B-5. There was no mortality when Fathead Minnow were tested against sample extracts from sites WD-3 and AM-3; *Daphnia magna* averaged 100% and 90% mortality, respectively, for those sites. It was not possible to test for significance of these data relative to control responses using Toxstat 3.3, because the data were neither normal nor homogeneous, even after all transformations were tried.

The LC50 for the cadmium chloride reference toxicant was estimated at 18.5 ug/L cadmium for *D. magna*, and 468 ug/L cadmium for Fathead Minnow. This is at the

Table 3. Results of toxicity testing using *Daphnia magna* and *Pimephales promelas* (Fathead Minnow) on eluates of soil samples from six Superfund Sites in Western Washington State. Results are expressed as percent survival.

Site	Description	Sample Number	*Daphnia magna*	Fathead Minnow
Site #1	WD-1A	07-8205	100%	100%
	WD-1B	07-8206	93.3%	
	WD-2A	07-8207	100%	100%
	WD-2B	07-8208	100%	
	WD-3A	07-8210	0%	100%
	WD-3B	07-8211	0%	
Site #3	L-1A	07-8212	96.7%	100%
	L-1B	07-8213	90%	
	L-2A	07-8214	100%	96.7%
	L-2B	07-8215	96.8%	
	L-3A	07-8217	0%	36.7%
	L-3B	07-8218	0%	
Site #2	CP-1A	07-8219	90%	100%
	CP-1B	07-8220	93.3%	
	CP-2A	07-8221	100%	100%
	CP-2B	07-8222	100%	
	CP-3A	07-8223	0%	0%
	CP-3B	07-8224	0%	

Table 4. Results of toxicity testing using *Daphnia magna* and *Pimephales promelas* (Fathead Minnow) on eluates of soil samples from six Superfund Sites in Eastern Washington State.

Site	Description	Sample Number	*Daphnia magna*	Fathead Minnow
Site #6	B-1A	10-8230	100%	100%
	B-1B	10-8231	100%	
	B-2A	10-8232	100%	100%
	B-2B	10-8233	100%	
	B-3A	10-8235	96.7%	100%
	B-3B	10-8236	93.3%	
	B-4A	10-8238	100%	100%
	B-4B	10-8239	96.7%	
	B-5A	10-8240	36.7%	0%
	B-5B	10-8241	76.7%	
Site #5	RA-1A	10-8242	96.7%	100%
	RA-1B	10-8243	96.7%	
	RA-~~2A~~ 3A	10-8244	100%	93.3%
	RA-~~2B~~ 3B	10-8245	96.7%	
	RA-~~3A~~ 2A	10-8247	100%	96.7%
	RA-~~3B~~ 2B	10-8248	100%	
Site #4	AM-1A	10-8249	100%	96.7%
	AM-1B	10-8250	100%	
	AM-2A	10-2851	80%	96.7%
	AM-2B	10-8252	83.3%	
	AM-3A	10-8254	0%	93.3%
	AM-3B	10-8255	20%	

high end of the range of values normally observed for *Daphnia magna* by this Laboratory. Because this laboratory's database for Fathead Minnow is based on daily renewal, it is no surprise that the LC50 estimate is considerably higher than that normally observed by this Laboratory for this organism. Copies of printouts from statistical analyses are in Appendix IV.

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ANALYSIS OF THE DEVELOPMENTAL TOXICITY OF AQUEOUS SOIL SAMPLE EXTRACTS FROM THE STATE OF WASHINGTON USING FROG EMBRYO TERATOGENESIS ASSAY-*XENOPUS* (FETAX)

INTRODUCTION

STOVER BIOMETRIC LABORATORIES, INC. was contracted to conduct development toxicity studies on aqueous extracts of soil samples collected by the State of Washington Department of Ecology using Frog Embryo Teratogenesis Assay-*Xenopus* (FETAX). FETAX is a 4-day whole-embryo, static-renewal developmental toxicity bioassay employing embryos of the South African clawed frog, *Xenopus laevis*. FETAX testing was conducted as a component of a battery of terrestrial and aquatic bioassay pilot tests to be used ultimately in the development of guidelines for addressing environmental protection at hazardous waste sites under the Model Toxics Control Act (MTCA). Testing was conducted as a partial fulfillment of Phase I (Bioassay Assessment) of the pilot program. Results from the first round of soil sampling are presented in this report.

TEST MATERIALS

Sampling of the soil was performed by Washington State Department of Ecology personnel. Samples were shipped by Washington State Department of Ecology personnel via commercial carrier on February 11, 1993. Nine soil samples were received by STOVER BIOMETRIC LABORATORIES, INC. on February 12, 1993. Sample temperatures upon receipt were all below 4°C. All samples were stored at <5°C until extracted.

Moisture Fraction Determination

The moisture fraction (MF) of each of the soil samples were determined immediately upon receipt by drying a 125 g aliquot of the soil sample in a crystalizing dish at 105°C for 24-hours. The MF was then calculated by subracting the final dry weight from the initial wet weight and dividing this result by the subsample weight (125 g).

TEST METHODS

Eluate Preparation

A weight of FETAX solution four times that of the dry weight of each soil sample was mixed together with each separate soil sample in a 1-l plastic cubitainer. Specifically, 100 g of soil (dry weight) was mixed with 400 g of FETAX solution. FETAX solution was prepared as described by Dawson and Bantle (1987) and Fort *et al.* (1988). Aqueous soil mixtures were then tumbled in a rotary extractor for 48-hours at 30±2 rpm and 22±2°C in the dark. Sample mixtures were prepared to minimize head space and thus, reduce the potential for volatilization. The tumbled samples were allowed to settle overnight in refrigerated storage. The samples were then centrifuged for approximately 20 minutes at 8,000 rpm until the supernatant was completely clear. The extract was then decanted and the pH and dissolved oxygen measured. Since none of the samples deviated from the acceptable pH range 6.5 - 9.0, the pH of the extracts were not adjusted. Enough eluate for initiating the tests were stored in a vented refrigerator at <5°C prior to initiating the FETAX tests. Additional 12 ml aliquots of the eluate samples were frozen at -20°C until used for test renewal. Prior to testing, dissolved oxygen, pH, conductivity, hardness, alkalinity, and ammonia-nitrogen were measured in each extract prepared. Dissolved oxygen and pH were measured daily prior to and after test renewal (waste).

FETAX Protocol

Animal Care & Breeding. *Xenopus* adult cure, breeding, and embryo collection were performed as described in the new ASTM Standard Guide (1991) for conducting FETAX.

Assay Protocol. FETAX was conducted as described in the new ASTM Standard Guide (1991) for conducting FETAX. A summary of FETAX test conditions is provided in Table 1. Groups of 25 embryos were placed in 60 mm covered, glass Petri dishes containing undiluted eluate samples. Each eluate sample was tested in triplicate. Three separate dishes of 25 embryos were exposed to FETAX solution alone and designated FETAX solution controls (negative controls). In addition, 4 dishes of 25 embryos were exposed to 6-aminonicotinamide, 2 sets at 5.5 mg/l (approximate EC50 [malformation]) and 2 sets at 2,500 mg/l (approximate 4-day LC50) as specified in the new ASTM Standard Guide (1991) for conducting FETAX and served as positive controls. Each treatment contained a total of 10 ml of solution. Embryos were cultured at 23.0±1.0°C.

TABLE 1
SUMMARY OF FROG EMBRYO TERATOGENESIS ASSAY-*XENOPUS* (FETAX)
TEST CONDITIONS

1.	Test type:	Static renewal
2.	Temperature:	23° ± 1°C
3.	Light quality:	Ambient laboratory illumination
4.	Light intensity:	10-20 uE/m²/s (50-100 ft-c) (ambient laboratory levels)
5.	Photoperiod:	12 hours light, 12 hours dark
6.	Test chamber size:	25 ml
7.	Test solution volume:	10 ml per replicate
8.	Renewal of test solutions:	Daily
9.	Age of test organisms:	Small cell blastulae (Stage 8-10)
10.	No. of larvae per chamber:	25
11.	No. of replicate test chambers per concentration:	3
12.	No. larvae per concentration:	75
13.	Test vessel randomization:	Randomization chart #3 was utilized for this test
14.	Feeding regime:	None
15.	Cleaning:	Siphoned daily, immediately before solution renewal
16.	Aeration:	None
17.	Dilution water:	FETAX Solution was prepared using E-PURE[R] deionized water and reagent grade chemicals.
18.	Extract concentrations:	100%, 0% (control)
19.	Test duration:	4 days

(continued)

TABLE 1
SUMMARY OF FROG EMBRYO TERATOGENESIS ASSAY-*XENOPUS* (FETAX)
TEST CONDITIONS
(CONTINUED)

20.	Endpoints:	Survival, malformation, growth (length)
21.	Test acceptability:	Mortality and malformation rates in control $\leq 10\%$ and $\leq 7\%$, respectively.

All solutions were changed every 24 hours of the 4-day test, dead embryos removed and recorded, and fresh solutions added. Following 4-days of exposure (stage 46 embryos), embryos were fixed in 3% formalin (pH=7.0) and the number of live malformed embryos were determined using a dissecting microscope.

Data Analysis. Mortality and malformation rates were determined for each sample tested, as well as, the positive and negative controls. Corrected mortality and malformation rates were calculated by adjusting for control mortality and malformation rates. Head-tail length of the surviving larvae was measured as an index of growth using an IBM-AT computer and Sigma Scan (Jandell Scientific, Corte Madera, CA) digitizing software.

RESULTS AND DISCUSSION

The effect of the soil extract samples on *Xenopus* development (survival, malformation and growth) is presented in Table 2. FETAX negative control (FETAX solution) mortality and malformation rates were 5.3% and 2.8%, respectively. Mortality rates in the 5.5 mg/l and 2,500 mg/l 6-aminonicotinamide positive control were 16.0% and 42.5%, and 64% and 100.0%, respectively.

The aqueous extract of soil sample CP-1A induced mortality and malformation rates of 9.3% and 5.6%, respectively. Mortality and abnormality rates for embryos exposed to an aqueous extract of soil sample CP-2A were 44.0% and 58.1%, respectively. An aqueous extract of sample CP-3A induced complete embryo lethality prior to 24-hours of exposure. All embryos exposed to CP-3A aqueous extract were arrested during or prior to neurulation suggesting that the toxicants may be inhibiting biochemical events important for neurulation, such as inhibition of microfilament activity.

An aqueous extract of soil sample WD-1A induced nominal rates of mortality and malformation of 2.7% and 1.4%, respectively. An aqueous extract of soil sample WD-2A induced mortality and malformation rates of 29.3% and 100.0%, respectively. Mortality and deformity rates of 49.3% and 100.0%, respectively, were observed with *Xenopus* exposed to an aqueous extract of soil sample WD-3A.

An aqueous extract of soil sample ~~3A~~ JC-1A induced nominal mortality and abnormality rates of 0% and 4.0%, respectively. Mortality and deformity rates of 29.3% and 28.3% were observed with embryos exposed to an aqueous extract of sample ~~3A~~ JC-2A. An aqueous extract

TABLE 2
EFFECT OF SOIL EXTRACT SAMPLES ON *XENOPUS* DEVELOPMENT USING FROG EMBRYO TERATOGENESIS ASSAY-*XENOPUS* (FETAX)

SAMPLE	MORTALITY		MALFORMATION		
	% Mortality (n)	% Corrected Mortality[1] (n)	% Malformation (n)	% Corrected Malformation[2] (n)	Mean Growth[3] (n)
FETAX Solution	5.3 (75)	-	2.8 (71)	-	89.7 (71)
6-AN (5.5 mg/l)	16.0 (50)	11.3 (50)	45.2 (42)	43.6 (42)	80.4 (42)
6-AN (2,500 mg/l)	64.0 (50)	63.0 (50)	100.0 (18)	100.0 (18)	84.7 (18)
CP-1A	9.3 (75)	4.2 (75)	5.6 (71)	2.9 (71)	85.8 (71)
CP-2A	44.0 (75)	40.9 (75)	58.1 (48)	56.9 (48)	87.9 (48)
CP-3A	100.0 (75)	100.0 (75)	-	-	-
WD-1A	2.7 (75)	0.0 (75)	1.4 (74)	0.0 (74)	85.3 (74)
WD-2A	29.3 (75)	25.3 (75)	56.6 (53)	55.3 (53)	87.4 (53)

(continued)

TABLE 2
EFFECT OF SOIL EXTRACT SAMPLES ON *XENOPUS* DEVELOPMENT USING FROG EMBRYO TERATOGENESIS ASSAY-*XENOPUS* (FETAX)
(CONTINUED)

SAMPLE	MORTALITY		MALFORMATION		
	% Mortality (n)	% Corrected Mortality[1] (n)	% Malformation (n)	% Corrected Malformation[2] (n)	Mean Growth[3] (n)
WD-3A	49.3 (75)	46.5 (75)	100.0 (38)	100.0 (38)	73.4 (38)
~~L-1A~~ JC-1A	0.0 (75)	0.0 (75)	4.0 (75)	1.2 (75)	88.7 (75)
~~L-2A~~ JC-2A	29.3 (75)	25.3 (75)	28.3 (53)	26.2 (53)	86.4 (53)
~~L-3A~~ JC-3A	50.7 (75)	47.9 (75)	43.2 (37)	41.6 (37)	85.7 (37)

1 Corrected mortality (%) = observed mortality (%) - negative control mortality/100 - negative control mortality (%).
2 Corrected malformation (%) = observed malformation (%) - negative control mortality/100 - negative control mortality (%).
3 Expressed in mm.

of soil sample JC-3A ~~L-3A~~ induced mortality and malformation rates of 50.7% and 43.2%, respectively.

A summary of the terata induced in both the negative (FETAX solution) and the positive (6-aminonicotinamide) controls, as well as, the aqueous soil extract treatments is provided in Table 3. A progressive occurence of similar types of malformations (or characteristic abnormalities) were generally observed with each subset of samples. Thus, the rates characteristic terata increased from negligible levels in the baseline site to increasingly more significant with increasing levels of contamination. Extracts of sample CP-1A induced nominal levels of gut miscoiling only. Extracts of sample CP-2A induced gut miscoiling, visceral and craniofacial edema, and skeletal kinking. The term skeletal kinking is used to differentiate from that of kinking caused by muscular contraction. Skeletal kinking, in contrast, typically involves defective development of the notocord and possibly myotomes.

An aqueous extract of samples WD-1A induced one case of gut miscoiling only. Aqueous extracts of soil samples WD-2A and WD-3A caused substantial miscoiling of the gut, visceral edema, skeletal kinking, microencephaly, and microophthalmia. In addition, embryos exposed to an extract of sample WD-3A also demonstrated significant visceral hemorrhage. Interestingly, gut miscoiling, visceral edema, and skeletal kinking have been found to be characteristic malformations induced by exposure of *Xenopus* to several heavy metal mixtures.

An aqueous extract of soil sample JC-1A ~~L-1A~~ induced nominal levels of slight gut miscoiling. An aqueous extract of samples JC-2A ~~L-2A~~ and JC-3A ~~L-3A~~ induced gut miscoiling, muscular kinking, mal-development of the eye, and hydroencephaly.

Initial physical/chemical water quality measurements are provided in Table 4. Each of the standard parameters measured were acceptable for the culture of *Xenopus* and did not deviate from those normally encountered with soil, sediment, or complex mixture testing. Daily dissolved oxygen and pH measurements (prior to and following [waste] renewal) are presented in Table 5. Again dissolved oxygen and pH values were suitable for the culture of *Xenopus* embryos and were similar to that normally observed. FETAX raw data sheets and Toxicity Test Soil Data Collection Sheets are presented as Appendices A and B, respectively. The sample chain of custody form is included as Appendix C.

TABLE 3
TERATA INDUCED IN *XENOPUS* BY EXPOSURE TO SOIL EXTRACTS

SAMPLE	TERATA INDUCED (number responding)
FETAX Solution	gut miscoiling (2)
6-AN (5.5 mg/l)	gut miscoiling (19), visceral edema (19), muscular kinking (19), microophthalmia (19), microencephaly (12), mouth defects (19)
6-AN (2,500 mg/l)	gut miscoiling (18), visceral edema (18), muscular kinking (18), microophthalmia (18), microencephaly (18), mouth defects (18)
CP-1A	gut miscoiling (4)
CP-2A	gut miscoiling (21), visceral and craniofacial edema (18), skeletal kinking (21)
WD-1A	gut miscoiling (1)
WD-2A	gut miscoiling (30), visceral edema (22), skeletal kinking (20), craniofacial defects (10), microophthalmia (6), microencephaly (22)
WD-3A	gut miscoiling (38), visceral edema (35), skeletal kinking (38), craniofacial defects (38), microophthalmia (38), microencephaly (38), hemorrhage (35)
~~1-1A~~ JC-1A	gut miscoiling (3)
~~1-2A~~ JC-2A	gut miscoiling (15), skeletal kinking (6), hydroencephaly (6)
~~1-3A~~ JC-3A	gut miscoiling (16), muscular kinking (10), eye defects (7), microencephaly (16)

TABLE 4
INITIAL PHYSICAL/CHEMICAL EXTRACT CHARACTERISTICS

SAMPLE	PARAMETER					
	Moisture Fraction (%)	Dissolved Oxygen (mg/l)	pH (s.u.)	Conductivity (μmhos/cm^2)	Hardness (mg/l as $CaCO_3$)	Alkalinity (mg/l as $CaCO_3$)
FETAX Solution	-	10.8	7.4	1673	120	60
CP-1A	5.8	10.2	7.8	1718	218	104
CP-2A	12.6	10.0	7.8	1816	284	150
CP-3A	13.9	9.9	7.8	1786	524	154
WD-1A	11.9	10.7	7.0	1581	140	26
WD-2A	18.2	9.8	6.6	1518	260	40
WD-3A	16.1	10.3	6.7	2566	120	16
~~EBA~~ JC-1A	8.6	10.0	7.2	1578	178	24
~~EBA~~ JC-2A	16.6	10.4	7.0	1528	154	18
~~EBA~~ JC-3A	20.5	10.0	7.0	1522	206	26

TABLE 5
DAILY DISSOLVED OXYGEN AND pH MEASUREMENT OF RENEWAL SAMPLES

Sample	Day 1				2				3				4			
	D.O.[1]		pH[2]		D.O.[1]		pH[2]		D.O.[1]		pH[2]		D.O.[1]		pH[2]	
	initial	final	initial	final	initial	final	initial	final	initial	final	initial	final	initial	final	initial	final
FETAX Solution	9.2	5.8	7.2	7.2	7.2	6.3	7.5	7.2	8.2	6.6	8.0	7.4	-	6.6	-	7.0
6-AN (5.5 mg/l)	8.2	8.2	7.7	7.4	8.2	6.0	7.6	7.3	8.3	6.8	7.5	7.0	-	6.2	-	7.3
6-AN (2,500 mg/l)	8.2	8.0	7.7	7.7	8.0	5.7	7.7	7.2	8.0	6.4	7.6	7.4	-	6.9	-	6.9
CP-1A	9.2	6.4	7.2	7.5	7.4	5.1	7.5	7.4	8.2	6.8	7.6	7.7	-	6.3	-	7.3
CP-2A	8.4	6.4	6.9	7.3	7.4	4.6	7.5	7.5	8.0	6.4	7.6	7.8	-	6.7	-	7.2
CP-3A	8.6	6.9	7.5	7.6	7.0	-	7.8	-	-	-	-	-	-	-	-	-
WD-1A	7.6	5.1	7.8	6.9	6.6	6.4	7.1	7.1	6.6	6.4	7.2	7.1	-	6.8	-	7.1
WD-2A	8.3	6.8	6.4	6.6	7.0	5.6	6.6	6.4	8.2	6.7	6.4	6.4	-	6.9	-	7.0
WD-3A	8.0	6.8	6.5	6.8	7.2	5.9	6.8	6.5	7.0	6.4	6.7	6.7	-	6.2	-	6.9
~~FETAX~~ JC-1A	9.3	6.6	6.7	7.1	7.0	5.1	7.5	6.8	7.6	6.8	7.4	7.2	-	6.9	-	7.2
~~6-AN~~ JC-2A	8.9	6.6	6.7	7.1	7.0	5.1	7.5	6.8	6.4	6.8	7.0	6.8	-	6.7	-	7.2
~~6-AN~~ JC-3A	8.7	6.4	6.9	7.0	7.0	5.2	7.1	6.0	8.6	6.2	7.2	7.0	-	7.0	-	7.2

1 Expressed as mg/l
2 Expressed as s.u.

TEST VALIDITY

FETAX solution negative controls induced mortality and malformation rates <7%. 6-aminonicotinamide positive controls induced mortality and malformation rates will within acceptable limits. Based on this data, the test data met or exceeded all test acceptance criteria.

CONCLUSIONS

Results from these studies indicated that each sample site induced a contaminant concentration-related increase the rates of mortality and malformation. Samples from the CP site tended to induce greater levels of embryo lethality, whereas the WD site samples demonstrated greater teratogenic potential (i.e., separation between mortality and malformation response rates). The JC site samples induced comparable rates of mortality and malformation. Results from this study indicated that FETAX was sensitive enough to detect developmental toxicants, yet robust enough to be suitable for aqueous soil extract testing. Results support the continued use of FETAX on this project and similar projects involving developmental toxicity hazard assessment.

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INTRODUCTION

STOVER BIOMETRIC LABORATORIES, INC. was contracted to conduct development toxicity studies on aqueous extracts of soil samples collected by the State of Washington Department of Ecology using Frog Embryo Teratogenesis Assay-*Xenopus* (FETAX). FETAX is a 4-day whole-embryo, static-renewal developmental toxicity bioassay employing embryos of the South African clawed frog, *Xenopus laevis*. FETAX testing was conducted as a component of a battery of terrestrial and aquatic bioassay pilot tests to be used ultimately in the development of guidelines for addressing environmental protection at hazardous waste sites under the Model Toxics Control Act (MTCA). Testing was conducted as a partial fulfillment of Phase I (Bioassay Assessment) of the pilot program. Results from the ~~first~~ 2nd round of soil sampling are presented in this report.

TEST MATERIALS

Sampling of the soil was performed by Washington State Department of Ecology personnel. Samples were shipped by Washington State Department of Ecology personnel via commercial carrier on March 4, 1993. Eleven soil samples were received by STOVER BIOMETRIC LABORATORIES, INC. on March 5, 1993. Sample temperatures upon receipt were all below 4°C. All samples were stored at <5°C until extracted.

Moisture Fraction Determination

The moisture fraction (MF) of each of the soil samples were determined immediately upon receipt by drying a 50 g aliquot of the soil sample in a crystalizing dish at 105°C for 24-hours. The MF was then calculated by subracting the final dry weight from the initial wet weight and dividing this result by the subsample weight (50 g).

TEST METHODS

Eluate Preparation

A weight of FETAX solution four times that of the dry weight of each soil sample was mixed together with each separate soil sample in a 1-l plastic cubitainer. Specifically, 200 g of soil (dry weight) was mixed with 800 g of FETAX solution. FETAX solution was prepared as described by Dawson and Bantle (1987) and Fort *et al.* (1988). Aqueous soil mixtures were then tumbled in a rotary extractor for 48-hours at 30±2 rpm and 22±2°C in the dark. Sample mixtures were prepared to minimize head space and thus, reduce the potential for volatilization. The tumbled samples were allowed to settle overnight in refrigerated storage. The samples were then centrifuged for approximately 20 minutes at 8,000 rpm until the supernatant was completely clear. The extract was then decanted and the pH and dissolved oxygen measured. With the exception of the AM-2A and AM-3A samples, none of the samples deviated from the acceptable pH range 6.5 - 9.0. The pH of the AM-2A and AM-3A samples was raised to 7.0 prior to testing with NaOH. Enough eluate for initiating the tests were stored in a vented refrigerator at <5°C prior to initiating the FETAX tests. Additional 12 ml aliquots of the eluate samples were frozen at -20°C until used for test renewal. Prior to testing, dissolved oxygen, pH, conductivity, hardness, alkalinity, and ammonia-nitrogen were measured in each extract prepared. Dissolved oxygen and pH were measured daily prior to and after test renewal (waste).

FETAX Protocol

Animal Care & Breeding. *Xenopus* adult cure, breeding, and embryo collection were performed as described in the new ASTM Standard Guide (1991) for conducting FETAX.

Assay Protocol. FETAX was conducted as described in the new ASTM Standard Guide (1991) for conducting FETAX. A summary of FETAX test conditions is provided in Table 1. Groups of 25 embryos were placed in 60 mm covered, glass Petri dishes containing undiluted eluate samples. Each eluate sample was tested in triplicate. Three separate dishes of 25 embryos were exposed to FETAX solution alone and designated FETAX solution controls (negative controls). In addition, 4 dishes of 25 embryos were exposed to 6-aminonicotinamide, 2 sets at 5.5 mg/l (approximate EC50 [malformation]) and 2 sets at 2,500 mg/l (approximate 4-day LC50) as specified in the new ASTM Standard Guide (1991) for conducting FETAX and served as positive controls. Each

TABLE 1
SUMMARY OF FROG EMBRYO TERATOGENESIS ASSAY-*XENOPUS* (FETAX)
TEST CONDITIONS

1.	Test type:	Static renewal
2.	Temperature:	23° ± 1°C
3.	Light quality:	Ambient laboratory illumination
4.	Light intensity:	10-20 uE/m²/s (50-100 ft-c) (ambient laboratory levels)
5.	Photoperiod:	12 hours light, 12 hours dark
6.	Test chamber size:	25 ml
7.	Test solution volume:	10 ml per replicate
8.	Renewal of test solutions:	Daily
9.	Age of test organisms:	Small cell blastulae (Stage 8-10)
10.	No. of larvae per chamber:	25
11.	No. of replicate test chambers per concentration:	3
12.	No. larvae per concentration:	75
13.	Test vessel randomization:	Randomization chart #3 was utilized for this test
14.	Feeding regime:	None
15.	Cleaning:	Siphoned daily, immediately before solution renewal
16.	Aeration:	None
17.	Dilution water:	FETAX Solution was prepared using E-PURE® deionized water and reagent grade chemicals.
18.	Extract concentrations:	100%, 0% (control)
19.	Test duration:	4 days

(continued)

TABLE 1
SUMMARY OF FROG EMBRYO TERATOGENESIS ASSAY-*XENOPUS* (FETAX)
TEST CONDITIONS
(CONTINUED)

20. Endpoints:	Survival, malformation, growth (length)
21. Test acceptability:	Mortality and malformation rates in control $\leq 10\%$ and $\leq 7\%$, respectively.

treatment contained a total of 10 ml of solution. Embryos were cultured at 23.0±1.0°C. All solutions were changed every 24 hours of the 4-day test, dead embryos removed and recorded, and fresh solutions added. Following 4-days of exposure (stage 46 embryos), embryos were fixed in 3% formalin (pH=7.0) and the number of live malformed embryos were determined using a dissecting microscope.

Data Analysis. Mortality and malformation rates were determined for each sample tested, as well as, the positive and negative controls. Corrected mortality and malformation rates were calculated by adjusting for control mortality and malformation rates. Head-tail length of the surviving larvae was measured as an index of growth using an IBM-AT computer and Sigma Scan (Jandell Scientific, Corte Madera, CA) digitizing software.

RESULTS AND DISCUSSION

The effects of the soil extract samples on *Xenopus* development (survival, malformation and growth) are presented in Table 2. FETAX negative control (FETAX solution) mortality and malformation rates were 5.3% and 2.8%, respectively. Mortality rates in the 5.5 mg/l and 2,500 mg/l 6-aminonicotinamide positive control were 8.0% and 60.9%, and 76.0% and 100.0%, respectively.

The aqueous extract of soil sample RA-1A induced nominal mortality and malformation rates of 0% and 2.7%, respectively. Mortality and abnormality rates for embryos exposed to an aqueous extract of soil sample ~~RA-2A~~ RA-3A were 0% and 26.7%, respectively. An aqueous extract of sample ~~RA-3A~~ RA-2A induced mortality and malformation rates of 0% and 70.7%, respectively.

An aqueous extract of soil sample AM-1A induced rates of mortality and malformation of 5.3% and 5.5%, respectively. An aqueous extract of soil sample AM-2A induced mortality and malformation rates of 41.3% and 100.0%, respectively. Mortality and deformity rates of 53.3% and 100.0%, respectively, were observed with *Xenopus* exposed to an aqueous extract of soil sample AM-3A.

An aqueous extract of soil sample B-1A induced no mortality or abnormality. Mortality and deformity rates of 0% and 20.0% were observed with embryos exposed to an aqueous extract of sample B-2A. An aqueous extract of soil sample B-3A induced mortality and

TABLE 2
EFFECT OF SOIL EXTRACT SAMPLES ON *XENOPUS* DEVELOPMENT USING FROG EMBRYO TERATOGENESIS ASSAY-*XENOPUS* (FETAX)

SAMPLE	MORTALITY		MALFORMATION		
	% Mortality (n)	% Corrected Mortality[1] (n)	% Malformation (n)	% Corrected Malformation[2] (n)	Mean Growth[3] (n)
FETAX Solution	0.0 (75)	-	1.3 (75)		92.2 (75)
6-AN (5.5 mg/l)	8.0 (50)	8.0 (50)	60.9 (42)	60.4 (46)	92.2 (46)
6-AN (2,500 mg/l)	76.0 (50)	76.0 (50)	100.0 (12)	100.0 (12)	78.5 (12)
RA-1A	0.0 (75)	0.0 (75)	2.7 (75)	1.4 (75)	87.2 (75)
RA-~~3~~ 3A	0.0 (75)	0.0 (75)	26.7 (75)	25.7 (75)	88.3 (75)
RA-~~2~~ 2A	0.0 (75)	0.0 (75)	70.7 (75)	70.3 (75)	88.5 (75)
AM-1A	5.3 (75)	5.3 (75)	5.5 (73)	4.3 (73)	87.3 (73)
AM-2A	41.3 (75)	41.3 (75)	100.0 (44)	100.0 (44)	64.6 (44)

(continued)

TABLE 2
EFFECT OF SOIL EXTRACT SAMPLES ON *XENOPUS* DEVELOPMENT USING FROG EMBRYO TERATOGENESIS ASSAY-*XENOPUS* (FETAX)
(CONTINUED)

SAMPLE	MORTALITY		MALFORMATION		Mean Growth[3] (n)
	% Mortality (n)	% Corrected Mortality[1] (n)	% Malformation (n)	% Corrected Malformation[2] (n)	
AM-3A	53.3 (75)	53.3 (75)	100.0 (35)	100.0 (35)	78.3 (35)
B-1A	0.0 (75)	0.0 (75)	0.0 (75)	0.0 (75)	86.0 (75)
B-2A	0.0 (75)	0.0 (75)	20.0 (75)	18.9 (75)	88.0 (75)
B-3A	25.3 (75)	25.3 (75)	76.8 (56)	76.5 (56)	85.5 (56)
B-4A	74.0 (75)	74.0 (75)	100.0 (38)	100.0 (38)	88.1 (38)
B-5A	80.0 (75)	80.0 (75)	100.0 (15)	100.0 (15)	68.7 (15)

1 Corrected mortality (%) = observed mortality (%) - negative control mortality/100 - negative control mortality (%).
2 Corrected malformation (%) = observed malformation (%) - negative control mortality/100 - negative control mortality (%).
3 Expressed in mm.

malformation rates of 25.3% and 76.8%, respectively. An aqueous extract of sample B-4A induced mortality and malformation rates of 74.0% and 100.0%, respectively. Mortality and abnormality rates of 80.0% and 100.0% respectively, were observed in embryos exposed to an aqueous extract of sample B-5A.

A summary of the terata induced in both the negative (FETAX solution) and the positive (6-aminonicotinamide) controls, as well as, the aqueous soil extract treatments is provided in Table 3. A progressive occurence of similar types of malformations (or characteristic abnormalities) were generally observed with each subset of samples. Thus, the rates characteristic terata increased from negligible levels in the baseline site to increasingly more significant with increasing levels of contamination. Extracts of sample RA-1A induced nominal levels of gut miscoiling only. Extracts of sample RA-3A induced gut miscoiling, craniofacial defects, and skeletal kinking. The term skeletal kinking is used to differentiate from that of kinking caused by muscular contraction. Skeletal kinking, in contrast, typically involves defective development of the notocord and possibly myotomes. Extracts of sample RA-3A induced gut miscoiling, craniofacial defects, visceral edema, skeletal kinking, anencephaly, and visceral hemorrhage.

An aqueous extract of samples AM-1A induced one case of gut miscoiling only. Aqueous extracts of soil samples AM-2A and AM-3A caused substantial miscoiling of the gut, visceral edema, skeletal kinking, microencephaly, visceral hemorrage, and microophthalmia. In addition, embryos exposed to an extract of sample WD-3A also demonstrated significant visceral hemorrhage. Interestingly, gut miscoiling, visceral edema, and skeletal kinking have been found to be characteristic malformations induced by exposure of *Xenopus* to several heavy metal mixtures.

An aqueous extract of soil sample B-2A induced gut miscoiling and microophthalmia. An aqueous extract of samples B-3A, B-4A, and B-5A induced gut miscoiling, mal-development of the eye, craniofacial defects, microencephaly, and visceral hemorrage.

Initial physical/chemical water quality measurements are provided in Table 4. Each of the standard parameters measured were acceptable for the culture of *Xenopus* and did not deviate from those normally encountered with soil, sediment, or complex mixture testing. Daily dissolved oxygen and pH measurements (prior to and following [waste] renewal) are presented in Table 5. Again dissolved oxygen and pH values were suitable for the culture of *Xenopus* embryos and were similar to that normally observed. FETAX raw data sheets

TABLE 3
TERATA INDUCED IN *XENOPUS* BY EXPOSURE TO SOIL EXTRACTS

SAMPLE	TERATA INDUCED (number responding)
FETAX Solution	gut miscoiling (1)
6-AN (5.5 mg/l)	gut miscoiling (28), visceral edema (28), muscular kinking (23), mouth defects (12)
6-AN (2,500 mg/l)	gut miscoiling (12), visceral edema (12), muscular kinking (12), microophthalmia (12), microencephaly (12), mouth defects (12)
RA-1A	gut miscoiling (2)
RA-3A	gut miscoiling (12), craniofacial defects (12), skeletal kinking (2), microencephaly (12)
RA-2A	gut miscoiling (50), craniofacial defects (50), visceral edema (12), skeletal kinking (10), anencephaly (52), hemorrhage (2)
AM-1A	gut miscoiling (4)
AM-2A	gut miscoiling (42), visceral edema (28), skeletal kinking (44), craniofacial defects (44), microophthalmia (10)
AM-3A	gut miscoiling (35), visceral edema (29), skeletal kinking (35), craniofacial defects (35), microophthalmia (35), microencephaly (2), hemorrhage (1)
B-2A	gut miscoiling (10), microophthalmia (15)
B-3A	gut miscoiling (43), eye defects (43), microencephaly (10), hemorrhage (2)
B-4A	gut miscoiling (38), craniofacial defects (20), microophthalmia (2), microencephaly (10), hemorrhage (1)
B-5A	gut miscoiling (15), craniofacial defects (15), microophthalmia (15), microencephaly (15), hemorrhage (15)

TABLE 4
INITIAL PHYSICAL/CHEMICAL EXTRACT CHARACTERISTICS

SAMPLE	PARAMETER					
	Moisture Fraction (%)	Dissolved Oxygen (mg/l)	pH (s.u.)	Conductivity (μmhos/cm^2)	Hardness (mg/l as $CaCO_3$)	Alkalinity (mg/l as $CaCO_3$)
FETAX Solution	-	8.6	7.9	2230	160	70
RA-1A	29.2	8.6	8.0	754	180	102
RA-~~2~~3A	22.2	8.4	8.3	2350	240	132
RA-~~3~~2A	26.6	8.4	8.3	2840	420	120
AM-1A	5.0	8.2	7.6	827	140	50
AM-2A	8.6	7.4	2.3	6310	<20	146
AM-3A	10.6	8.2	3.8	2550	260	290
B-1A	23.8	8.0	6.8	1157	180	40
B-2A	11.4	7.8	8.0	1399	200	94
B-3A	22.4	7.8	7.1	1453	220	68
B-4A	11.4	7.6	8.0	1844	260	100
B-5A	19.4	8.6	7.7	1944	360	140

TABLE 5
DAILY DISSOLVED OXYGEN AND pH MEASUREMENT OF RENEWAL SAMPLES

Sample	Day 1				2				3				4			
	D.O.[1]		pH[2]		D.O.[1]		pH[2]		D.O.[1]		pH[2]		D.O.[1]		pH[2]	
	initial	final	initial	final	initial	final	initial	final	initial	final	initial	final	initial	final	initial	final
FETAX Solution	8.6	8.0	7.8	7.6	9.0	6.9	7.8	7.5	8.6	8.2	7.9	7.6	-	8.1	-	7.6
6-AN (5.5 mg/l)	7.5	8.4	7.8	7.7	7.6	7.2	7.9	7.7	7.7	7.6	7.9	7.8	-	8.1	-	7.5
6-AN (2,500 mg/l)	7.5	8.6	7.9	7.7	7.6	7.3	7.9	7.7	7.8	7.6	7.9	7.9	-	7.1	-	7.4
RA-1A	8.6	7.6	7.7	7.7	8.6	5.8	7.7	8.2	5.8	8.0	8.2	7.9	-	8.0	-	7.8
RA-~~2~~3A	8.4	7.6	8.1	7.8	8.4	5.5	8.1	8.5	5.5	8.1	8.5	8.0	-	8.3	-	7.9
RA-~~3~~2A	8.4	7.6	8.2	7.7	8.4	5.6	8.2	8.4	5.6	8.2	8.4	8.0	-	8.4	-	7.8
AM-1A	8.2	8.1	7.6	7.5	8.2	5.6	7.6	7.8	5.6	8.3	7.8	7.9	-	8.6	-	7.7
AM-2A	7.4	8.1	7.0	8.0	7.2	6.6	7.2	7.3	5.2	6.1	7.0	8.0	-	6.2	-	7.8
AM-3A	8.2	7.7	7.0	6.6	6.2	7.2	6.9	7.9	6.0	8.1	7.2	7.5	-	8.0	-	7.4
B-1A	8.0	8.2	7.5	7.3	7.5	7.4	7.4	7.4	6.1	8.2	7.1	7.5	-	8.6	-	7.4
B-2A	7.8	5.6	7.8	8.4	7.3	7.3	8.3	7.5	5.6	8.1	8.4	7.7	-	8.2	-	7.7
B-3A	7.8	6.0	7.3	7.7	7.1	6.9	7.6	7.4	6.0	8.0	7.7	7.6	-	8.3	-	7.6
B-4A	7.6	5.6	7.8	9.0	7.2	7.3	8.2	7.5	5.8	8.1	9.0	7.8	-	8.2	-	7.7
B-5A	5.6	4.8	7.8	8.2	6.2	4.9	8.3	7.4	4.8	6.3	8.2	7.7	-	7.3	-	7.7

1 Expressed as mg/l.
2 Expressed as s.u.

and Toxicity Test Soil Data Collection Sheets are presented as Appendices A and B, respectively. The sample chain of custody form is included as Appendix C.

TEST VALIDITY

FETAX solution negative controls induced mortality and malformation rates <7%. The 6-aminonicotinamide positive controls induced mortality and malformation rates well within acceptable limits. Based on this data, the test data met or exceeded all test acceptance criteria.

CONCLUSIONS

Results from these studies indicated that each sample site induced a contaminant concentration-related increase in the rates of mortality and malformation. Samples from each of the sites demonstrated teratogenic potential (i.e., separation between mortality and malformation response rates). The AM and B sites also induced progressing levels of embryolethality, whereas the RA site did not induce lethal effects. Results from this study indicated that FETAX was sensitive enough to detect developmental toxicants, yet robust enough to be suitable for aqueous soil extract testing. Results support the continued use of FETAX on this project and similar projects involving developmental toxicity hazard assessment.

REFERENCES

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Summary of Bioassay Results

Table C1: Results of Daphnia magna 48-hour toxicity testing for the Soil Bioassay Pilot Study (Results expressed as percent survival).

I. Western Washington

Site= Negative Control

Station	Replicate No. 1	2	3	4	5	6	Mean	Min	Max	S	C.V.	RPD	N
C1	100	100	100	-	-	-	100	100	100	0.0	0.00	0.0	3

Site #1= Metals

Station	Replicate No. 1	2	3	4	5	6	Mean	Min	Max	S	C.V.	RPD	N
WD1	100	100	100	90	100	90	97	90	100	5.2	0.05	10.3	6
WD2	100	100	100	100	100	100	100	100	100	0.0	0.00	0.0	6
WD3	0	0	0	0	0	0	0	0	0	0.0	-	0.0	6
								Site Mean		1.7	0.00	3.4	(18)

Site #2= Creosote

Station	Replicate No. 1	2	3	4	5	6	Mean	Min	Max	S	C.V.	RPD	N
CP1	80	90	100	80	100	100	92	80	100	9.8	0.11	21.8	6
CP2	100	100	100	100	100	100	100	100	100	0.0	0.00	0.0	6
CP3	0	0	0	0	0	0	0	0	0	0.0	-	0.0	6
								Site Mean		3.3	0.00	7.3	(18)

Site #3= Petroleum Products

Station	Replicate No. 1	2	3	4	5	6	Mean	Min	Max	S	C.V.	RPD	N
JC1	100	90	100	90	90	90	93	90	100	5.2	0.06	10.7	6
JC2	100	100	10	100	100	90	83	10	100	36.1	0.43	108.0	6
JC3	0	0	0	0	0	0	0	0	0	0.0	-	0.0	6
								Site Mean		13.8	0.00	39.6	(18)

S=Standard Deviation of the Sample
C.V.=Coefficient of Variation
RPD=Relative percent difference (range of responses/mean response)
( )=Total number of replicates

Table C1 (continued): Results of Daphnia magna 48-hour toxicity testing for the Soil Bioass Study (Results expressed as percent survival).

II. Eastern Washington

Site= Negative Control

Station	Replicate No. 1	2	3	4	5	6	Mean	Min	Max	S	C.V.	RPD	N
C1	100	100	100	-	-	-	100	100	100	0.0	0.00	0.0	3

Site #4= Metals

Station	Replicate No. 1	2	3	4	5	6	Mean	Min	Max	S	C.V.	RPD	N
AM1	100	100	100	100	100	100	100	100	100	0.0	0.00	0.0	6
AM2	60	100	80	70	90	90	82	60	100	14.7	0.18	49.0	6
AM3	0	0	0	20	20	20	10	0	20	11.0	1.10	200.0	6
								Site Mean		8.6	0.10	25.0	(18)

Site #5= Pesticides

Station	Replicate No. 1	2	3	4	5	6	Mean	Min	Max	S	C.V.	RPD	N
RA1	90	100	100	100	100	90	97	90	100	5.2	0.05	10.3	6
RA2	100	100	100	100	100	100	100	100	100	0.0	0.00	0.0	6
RA3	100	100	100	90	100	100	98	90	100	4.1	0.04	10.2	6
								Site Mean		3.1	0.03	6.8	(18)

Site #6= Petroleum Products

Station	Replicate No. 1	2	3	4	5	6	Mean	Min	Max	S	C.V.	RPD	N
B1	100	100	100	100	100	100	100	100	100	0.0	0.00	0.0	6
B2	100	100	100	100	100	100	100	100	100	0.0	0.00	0.0	6
B3	90	100	100	80	100	100	95	80	100	8.4	0.09	21.1	6
B4	100	100	100	100	90	100	98	90	100	4.1	0.04	10.2	6
B5	40	30	40	100	70	60	57	30	100	25.8	0.46	123.5	6
S=Standard Deviation of the Sample								Site Mean		7.7	0.12	31.0	(30)

C.V.=Coefficient of Variation

RPD=Relative percent difference (range of responses/mean response)

( )=Total number of replicates

	S	C.V.	RPD	N
Overall Mean	6.3	0.04	18.8	(120)

Table C2: Results of 14-day Earthworm (Eisenia fotetida) toxicity testing for the Soil Bioassay Pilot Study (Results expressed as percent survival).

I. Western Washington

Site= Negative Control

Station	Replicate 1	2	3	4	5	6	Mean	Min	Max	S	C.V.	RPD	N
C1	100	100	100	100	100	100	100	100	100	0.0	0.00	0.0	6

Site #1- Metals

Station	Replicate 1	2	3	4	5	6	Mean	Min	Max	S	C.V.	RPD	N
WD1	100	100	100	100	100	100	100	100	100	0.0	0.00	0.0	6
WD2	100	100	100	-	-	-	100	100	100	0.0	0.00	0.0	3
WD3	0	0	0	0	0	0	0	0	0	0.0	-	0.0	6
								Site Mean		0.0	0.00	0.0	(15)

Site #2- Creosote

Station	Replicate 1	2	3	4	5	6	Mean	Min	Max	S	C.V.	RPD	N
CP1	100	90	100	90	100	100	97	90	100	5.2	0.05	10.3	6
CP2	90	100	100	100	100	100	98	90	100	4.1	0.04	10.2	6
CP3	0	0	0	0	0	0	0	0	0	0.0	-	0.0	6
								Site Mean		3.1	0.03	6.8	(18)

Site #3- Petroleum Products

Station	Replicate 1	2	3	4	5	6	Mean	Min	Max	S	C.V.	RPD	N
JC1	90	100	100	100	100	90	97	90	100	5.2	0.05	10.3	6
JC2	100	80	90	100	80	90	90	80	100	8.9	0.10	22.2	6
JC3	0	0	0	0	0	0	0	0	0	0.0	-	0.0	6
								Site Mean		4.7	0.05	10.9	(18)

S=Standard Deviation
C.V.=Coefficient of Variation
RPD=Relative Percent Difference
( )=Total number of replicates

Table C2 (continued): Results of 14-day Earthworm (Eisenia fotetida) toxicity testing for the Soil Bioassay Pilot Study (Results expressed as percent survival).

II. Eastern Washington

Site= Negative Control

Station	Replicate 1	2	3	4	5	6	Mean	Min	Max	S	C.V.	RPD	N
C1	100	100	100	100	100	100	100	100	100	0.0	0.00	0.0	6

Site #4- Metals

Station	Replicate 1	2	3	4	5	6	Mean	Min	Max	S	C.V.	RPD	N
AM1	100	100	100	70	100	90	93	70	100	12.1	0.13	32.1	6
AM2	100	100	100	100	100	100	100	100	100	0.0	0.00	0.0	6
AM3	90	100	80	90	100	100	93	80	100	8.2	0.09	21.4	6
								Site Mean		6.8	0.07	17.9	(18)

Site #5- Pesticides

Station	Replicate 1	2	3	4	5	6	Mean	Min	Max	S	C.V.	RPD	N
RA1	100	100	100	100	100	100	100	100	100	0.0	0.00	0.0	6
RA2	90	100	90	100	90	100	95	90	100	5.5	0.06	10.5	6
RA3	100	90	100	100	100	100	98	90	100	4.1	0.04	10.2	6
								Site Mean		3.2	0.03	6.9	(18)

Site #6- Petroleum Products

Station	Replicate 1	2	3	4	5	6	Mean	Min	Max	S	C.V.	RPD	N
B1	100	90	100	100	100	100	98	90	100	4.1	0.04	10.2	6
B2	100	100	100	100	100	100	100	100	100	0.0	0.00	0.0	6
B3	100	100	100	100	100	100	100	100	100	0.0	0.00	0.0	6
B4	100	100	90	100	90	100	97	90	100	5.2	0.05	10.3	6
B5	0	0	90	0	0	40	22	0	90	37.1	1.71	415.4	6
								Site Mean		14.1	0.59	141.9	(30)

S=Standard Deviation
C.V.=Coefficient of Variation
RPD=Relative Percent Difference
( )=Total number of replicates

	S	C.V.	RPD	N
Overall Mean	5.3	0.13	30.7	(117)

Table C3: Results of 14-day Plant Vigor (Lactuca sativa) toxicity testing for the Soil Bioassay Pilot Study (Results expressed as biomass in mg).

I. Western Washington

Site= Negative Control

	Replicate												
Station	1	2	3	4	5	6	Mean	Min	Max	S	C.V.	RPD	N
C1	25.7	17.7	25.4	18.8	54.1	32	29	18	54	13.4	0.46	125.7	6

Site #1- Metals

	Replicate												
Station	1	2	3	4	5	6	Mean	Min	Max	S	C.V.	RPD	N
WD1	19.6	0.5	16.7	10.7	16.2	5.2	11	1	20	7.4	0.65	166.3	6
WD2	15.7	7.5	25.2	-	-	-	16	8	25	8.9	0.55	109.7	3
WD3	14.7	3.7	4.3	11.5	12.2	22.3	11	4	22	6.9	0.61	162.4	6
								Site Mean		7.7	0.60	146.2	(15)

Site #2- Creosote

	Replicate												
Station	1	2	3	4	5	6	Mean	Min	Max	S	C.V.	RPD	N
CP1	8.3	27.1	23.7	2.4	3.7	17.8	14	2	27	10.5	0.76	178.6	6
CP2	5.1	18.4	2.2	1	3.7	7.6	6	1	18	6.3	1.00	274.7	6
CP3	0	0	2.8	0	6.9	2.5	2	0	7	2.7	1.34	339.3	6
								Site Mean		6.5	1.03	264.2	(18)

Site #3- Petroleum Products

	Replicate												
Station	1	2	3	4	5	6	Mean	Min	Max	S	C.V.	RPD	N
JC1	69.7	52.3	0	34.1	3.9	40.7	33	0	70	27.3	0.81	208.4	6
JC2	7.6	19.1	0.9	7.4	10.6	4.4	8	1	19	6.2	0.75	218.4	6
JC3	22.6	15.9	4.8	0	18.6	25.9	15	0	26	10.2	0.70	177.0	6
								Site Mean		14.6	0.75	201.3	(18)

S=Standard Deviation of the Sample
C.V.=Coefficient of Variation
RPD=Relative Percent Difference
( )=Total Number of Replicates

Table C3 (continued): Results of 14-day Plant Vigor (Lactuca sativa) toxicity testing for the Soil Bioassay Pilot Study (Results expressed as biomass in mg).

II. Eastern Washington

Site= Negative Control

Station	Replicate 1	2	3	4	5	6	Mean	Min	Max	S	C.V.	RPD	N
C1	147.2	90.7	83.7	43.5	60.6	69.3	83	44	147	35.9	0.43	125.7	6

Site #4- Metals

Station	Replicate 1	2	3	4	5	6	Mean	Min	Max	S	C.V.	RPD	N
AM1	121	109.8	105.1	77.2	85	70.8	95	71	121	20.0	0.21	52.9	6
AM2	84.2	94	154.7	136.3	77.4	120.3	111	77	155	30.9	0.28	69.5	6
AM3	209.2	58.8	130.5	116.7	155.2	125.2	133	59	209	49.3	0.37	113.4	6
								Site Mean		33.4	0.29	78.6	(18)

Site #5- Pesticides

Station	Replicate 1	2	3	4	5	6	Mean	Min	Max	S	C.V.	RPD	N
RA1	89.5	106.6	66.4	64	82.5	97.2	84	64	107	16.9	0.20	50.5	6
RA2	58.4	22.8	51.5	14.7	3.7	6.1	26	4	58	23.4	0.89	208.8	6
RA3	12.2	5.9	4.1	10.1	16.2	6.6	9	4	16	4.5	0.49	131.8	6
								Site Mean		14.9	0.53	130.3	(18)

Site #6- Petroleum Products

Station	Replicate 1	2	3	4	5	6	Mean	Min	Max	S	C.V.	RPD	N
B1	69.9	54.7	64	82.6	52.8	85.7	68	53	86	13.8	0.20	48.2	6
B2	75.9	35.2	238.3	68.2	54.6	58.2	88	35	238	74.7	0.85	229.8	6
B3	68.2	54.6	58.2	45.3	13.7	70	52	14	70	20.7	0.40	109.0	6
B4	95.3	19.7	14.6	32.5	75.1	99.5	56	15	100	38.4	0.68	151.3	6
B5	20.3	15.6	16.4	4.5	17.8	21.1	16	5	21	6.0	0.38	104.1	6
S=Standard Deviation of the Sample								Site Mean		21.7	0.49	121.4	(30)

C.V.=Coefficient of Variation

RPD=Relative Percent Difference

( )=Total Number of Replicates

	S	C.V.	RPD	N
Overall Mean	16.5	0.61	157.0	(117)

Table C4: Results of 48-hour Fathead Minnow (Pimephales promelas) toxicity testing for the Soil Bioassay Pilot Study (Results expressed as percent survival).

I. Western Washington

Site= Negative Control

Station	Replicate 1	2	3	Mean	Min	Max	S	C.V.	RPD	N
C1	100	100	100	100	100	100	0.0	0.00	0.0	3

Site #1- Metals

Station	Replicate 1	2	3	Mean	Min	Max	S	C.V.	RPD	N
WD1	100	100	100	100	100	100	0.0	0.00	0.0	3
WD2	100	100	100	100	100	100	0.0	0.00	0.0	3
WD3	100	100	100	100	100	100	0.0	0.00	0.0	3
					Site Mean		0.0	0.00	0.0	(9)

Site #2- Creosote

Station	Replicate 1	2	3	Mean	Min	Max	S	C.V.	RPD	N
CP1	100	100	100	100	100	100	0.0	0.00	0.0	3
CP2	100	100	100	100	100	100	0.0	0.00	0.0	3
CP3	0	0	0	0	0	0	0.0	-	0.0	3
					Site Mean		0.0	0.00	0.0	(9)

Site #3- Petroleum Products

Station	Replicate 1	2	3	Mean	Min	Max	S	C.V.	RPD	N
JC1	100	100	100	100	100	100	0.0	0.00	0.0	3
JC2	100	100	90	97	90	100	5.8	0.06	10.3	3
JC3	0	70	40	37	0	70	35.1	0.96	190.9	3
					Site Mean		13.6	0.34	67.1	(9)

S=Standard Deviation of the Sample
C.V.=Coefficient of Variation
RPD=Relative Percent Difference
( )=Total Number of Replicates

II. Eastern Washington

Site= Negative Control

Station	Replicate 1	2	3	Mean	Min	Max	S	C.V.	RPD	N
C1	100	100	100	100	100	100	0.0	0.00	0.0	3
C2	70	100	70	80	70	100	17.3	0.22	37.5	3
C3	100	100	100	100	100	100	0.0	0.00	0.0	3

Site #4- Metals

Station	Replicate 1	2	3	Mean	Min	Max	S	C.V.	RPD	N
AM1	100	100	90	97	90	100	5.8	0.06	10.3	3
AM2	100	100	90	97	90	100	5.8	0.06	10.3	3
AM3	90	100	90	93	90	100	5.8	0.06	10.7	3
					Site Mean		5.8	0.06	10.5	(9)

Site #5- Pesticides

Station	Replicate 1	2	3	Mean	Min	Max	S	C.V.	RPD	N
RA1	100	100	100	100	100	100	0.0	0.00	0.0	3
RA2	100	100	90	97	90	100	5.8	0.06	10.3	3
RA3	80	100	100	93	80	100	11.5	0.12	21.4	3
					Site Mean		5.8	0.06	10.6	(9)

Site #6- Petroleum Products

Station	Replicate 1	2	3	Mean	Min	Max	S	C.V.	RPD	N
B1	100	100	100	100	100	100	0.0	0.00	0.0	3
B2	100	100	100	100	100	100	0.0	0.00	0.0	3
B3	100	100	100	100	100	100	0.0	0.00	0.0	3
B4	100	100	100	100	100	100	0.0	0.00	0.0	3
B5	0	0	0	0	0	0	0.0	-	0.0	3
					Site Mean		0.0	0.0	0.0	(15)

	S	C.V.	RPD	N
Overall Mean	4.2	0.08	14.7	(60)

Table C5: Results of 96-hour FETAX (Xenopus) toxicity testing for the Soil Bioassay Pilot Study (Results expressed as percent survival).

I. Western Washington

Site= Negative Control

Station	Replicate 1	2	3	Mean	Min	Max	S	C.V.	RPD	N
C1	100	92	92	95	92	100	4.6	0.05	8.5	3

Site #1- Metals

Station	Replicate 1	2	3	Mean	Min	Max	S	C.V.	RPD	N
WD1	92	100	100	97	92	100	4.6	0.05	8.2	3
WD2	72	72	68	71	68	72	2.3	0.03	5.7	3
WD3	52	48	52	51	48	52	2.3	0.05	7.9	3
					Site Mean		3.1	0.04	7.3	(9)

Site #2- Creosote

Station	Replicate 1	2	3	Mean	Min	Max	S	C.V.	RPD	N
CP1	88	92	92	91	88	92	2.3	0.03	4.4	3
CP2	60	60	48	56	48	60	6.9	0.12	21.4	3
CP3	0	0	0	0	0	0	0.0	-	0.0	3
					Site Mean		3.1	0.05	8.6	(9)

Site #3- Petroleum Products

Station	Replicate 1	2	3	Mean	Min	Max	S	C.V.	RPD	N
JC1	100	100	100	100	100	100	0.0	0.00	0.0	3
JC2	64	76	72	71	64	76	6.1	0.09	17.0	3
JC3	48	52	48	49	48	52	2.3	0.05	8.1	3
					Site Mean		2.8	0.04	8.4	(9)

S=Standard Deviation of the Sample
C.V.=Coefficient of Variation
RPD=Relative Percent Difference
( )=Total Number of Replicates

II. Eastern Washington

Site= Negative Control

Station	Replicate 1	2	3	Mean	Min	Max	S	C.V.	RPD	N
C1	100	100	100	100	100	100	0.0	0.00	0.0	3

Site #4- Metals

Station	Replicate 1	2	3	Mean	Min	Max	S	C.V.	RPD	N
AM1	92	96	96	95	92	96	2.3	0.02	4.2	3
AM2	52	60	64	59	52	64	6.1	0.10	20.5	3
AM3	60	40	40	47	40	60	11.5	0.25	42.9	3
					Site Mean		6.7	0.13	22.5	(9)

Site #5- Pesticides

Station	Replicate 1	2	3	Mean	Min	Max	S	C.V.	RPD	N
RA1	100	100	100	100	100	100	0.0	0.00	0.0	3
RA2	100	100	100	100	100	100	0.0	0.00	0.0	3
RA3	100	100	100	100	100	100	0.0	0.00	0.0	3
					Site Mean		0.0	0.00	0.0	(9)

Site #6- Petroleum Products

Station	Replicate 1	2	3	Mean	Min	Max	S	C.V.	RPD	N
B1	100	100	100	100	100	100	0.0	0.00	0.0	3
B2	100	100	100	100	100	100	0.0	0.00	0.0	3
B3	68	76	80	75	68	80	6.1	0.08	16.1	3
B4	60	40	52	51	40	60	10.1	0.20	39.5	3
B5	0	24	36	20	0	36	18.3	0.92	180.0	3
					Site Mean		6.9	0.24	47.1	(15)

	S	C.V.	RPD	N
Overall Mean	3.8	0.08	15.6	(60)

Appendix D

Tentatively Identified Organics in Soils from Sites 2,3, and 6

Table D1: Summary of tentatively identified semivolatile organics in soils from site #2 (creosote) for the Soil Bioassay Pilot Study (ug/kg, dry).

Location	Western Washington Site #2		
Station Conc. Range	CP-1 Background	CP-2 Medium	CP-3 High
1-Ethenyl-2-methyl-benzene	-	-	9400
1-Ethenyl-4-methyl-benzene	-	-	19000
1,2,3,4-tetramethyl benzene	-	-	4900
1-(2,4-cyclopentadiene)benzene	-	-	180000
1-Methyl anthracene	-	-	250000
2-Methyl anthracene	-	480	180000
2-Ethyl anthracene	-	-	36000
1,3-Dimethyl naphthalene	-	-	37000
1,4-Dimethyl naphthalene	-	-	12000
1,5-Dimethyl naphthalene	-	400	100000
1,6-Dimethyl naphthalene	-	-	74000
1,7-Dimethyl naphthalene	-	170	-
1,8-Dimethyl naphthalene	-	-	220000
2,3-Dimethyl naphthalene	-	210	77000
2,6-Dimethyl naphthalene	-	-	140000
2,7-Dimethyl naphthalene	-	-	100000
1,4,5-Trimethyl naphthalene	-	-	33000
1,4,6-Trimethyl naphthalene	-	-	41000
1,6,7-Trimethyl naphthalene	-	-	54000
2,3,6-Trimethyl naphthalene	-	-	38000
1-Ethyl naphthalene	-	-	78000
2-Ethenyl naphthalene	-	-	150000
2-(1-methylethyl)naphthalene	-	-	64000
1-Phenyl naphthalene	-	-	60000
2-Phenylnaphthalene	-	-	150000
Benzo(g,h,i)fluoranthene	-	560	-
1-Methyl phenanthrene	-	-	150000
2-Methyl phenanthrene	-	-	99000
9-Methyl phenanthrene	-	-	120000
2,5-Dimethyl phenanthrene	-	-	59000
4H-Cyclopenta(def)phenanthrene	-	1100	410000
3-Methyl phenanthrene	-	360	-
4,5 Methylene-9,10-phenanthrene	190	-	-
9,10-Dihydro phenanthrene	-	-	28000
2-Methyl-9H-fluorene	-	-	67000
4-Methyl-9H-fluorene	-	-	59000
11H-Benzo(a)fluorene	-	420	110000
11H-Benzo(b)fluorene	-	340	74000
9H-Fluoren-9-one	-	550	-
1-Methyl pyrene	-	-	24000
4-Methyl pyrene	-	410	-
Cyclopenta(cd)pyrene	-	640	-
Benzo(e)pyrene	-	1500	57000

All concentrations are reported as estimated values, based on presumptive evidence of material
-=Not detected at unspecified detection limit

Table D1 (continued): Summary of tentatively identified semivolatile organic in soils from site #2 (creosote) for the Soil Bioassay Pilot Study (ug/kg, dry).

Location	Western Washington Site #2		
Station Conc. Range	CP-1 Background	CP-2 Medium	CP-3 High
2,3-Dihydro-1-methylindene	-	-	8600
Dibenzothiophene	-	-	180000
Benzo(b)thiophene	-	430	29000
5-Methyl benzo(b)thiophene	-	-	25000
Benzo(b)naptho(1,2-d)thiophene	-	410	-
4-hydroxy-3-methyl benzaldehyde	-	260	-
1,1-Biphenyl	-	150	87000
2-Methyl-1,1-biphenyl	-	-	50000
4-Methyl-1,1-biphenyl	-	-	92000
2,3-Dimethyl-1,1-biphenyl	-	-	24000
Benzo(b)naptho(2,3-d)furan	-	-	38000
4-Methyl dibenzofuran	-	-	150000
1H-Indole	-	240	-
Stigmast-4-en-3-one	-	1500	-
Benzeneacetonitrile	-	340	-
Isoguinoline	-	-	38000
2-Methyl quinoline	-	-	29000
Benzo(f)quinoline	-	-	41000
3-Phenyl-2-propenenitrile	-	-	24000

All concentrations are reported as estimated values, based on presumptive evidence of mate
-=Not detected at unspecified detection limit

Table D2: Summary of tentatively identified semivolatile organics analyses of soils from petroleum products sites #3 and #6 for the Soil Bioassay Pilot Study (ug/kg, dry).

Location	Western Washington Site #3			Eastern Washington Site #6				
Station Conc. Range	JC-1 Background	JC-2 Medium	JC-3 High	B-1 Background	B-2 Med(Gas)	B-3 Med(Diesel)	B-4 High(Gas)	B-5 High(Diesel)
1-Ethyl-2-methyl benzene	–	990	58000	–	–	–	–	–
1-Ethyl-3-methyl benzene	–	1300	140000	–	–	–	–	–
1-Ethyl-2,3 dimethylbenzene	–	1200	94000	–	160	–	–	–
1-Ethyl-2,4 dimethylbenzene	–	–	46000	–	190	–	–	–
1-Ethyl-3,5 dimethylbenzene	–	2100	29000	–	250	–	–	–
4-Ethyl-1,2-dimethyl benzene	–	550	73000	–	–	–	–	–
2-Ethyl-1,3-dimethyl benzene	–	2000	26000	–	–	–	–	–
2-Ethyl-1,4-dimethyl benzene	–	3500	41000	–	–	–	–	–
1,3-Diethyl-5-methyl benzene	–	1200	–	–	–	–	–	–
1,4-Diethyl benzene	–	3700	16000	–	–	–	–	–
1-Methyl-2-propyl benzene	–	3100	29000	–	–	–	–	–
1-Methyl-3-propyl benzene	–	4500	53000	–	–	–	–	–
1-Methyl-4-propyl benzene	–	990	16000	–	–	–	–	–
(1,1-Dimethylpropyl) benzene	–	1000	25000	–	–	–	–	–
1,2-Dimethyl benzene	–	–	130000	–	–	–	–	–
1,4-Dimethyl benzene	–	–	100000	–	240	–	–	–
1,2,3-Trimethyl benzene	–	2700	110000	–	–	–	–	–
1,2,4 Trimethylbenzene	–	1700	72000	190	180	–	–	–
1,3,5-Trimethylbenzene	–	2600	120000	–	–	–	–	–
1,2,3,4 Tetramethylbenzene	–	1100	–	240	240	–	–	–
1,2,3,5 Tetramethylbenzene	–	2200	19000	–	300	–	–	–
1,2,4,5 Tetramethylbenzene	–	4500	–	–	–	–	–	–
1-Propenyl benzene	–	–	36000	–	–	–	–	–
1,4-dipropylbenzene	–	–	–	–	200	–	–	–
1-Ethyl naphthalene	–	–	2600	–	–	–	–	–
1,2-Dimethyl naphthalene	–	270	410	–	–	–	–	–
1,3-Dimethyl naphthalene	–	–	3500	–	–	–	–	6600
1,5-Dimethylnaphthalene	–	790	3000	–	–	–	–	–
1,6-Dimethylnaphthalene	–	–	22000	–	200	–	–	6100
1,7-Dimethylnaphthalene	–	810	–	–	–	–	–	–
1,8-Dimethylnaphthalene	–	580	–	–	–	–	–	–
2,3-Dimethylnaphthalene	–	470	–	–	–	–	–	–
2,6-Dimethylnaphthalene	–	690	–	–	–	–	–	–
2,7-Dimethylnaphthalene	–	–	4300	–	–	–	–	–
1,3,6-Trimethylnaphthalene	–	–	–	–	–	–	–	3200
1,4,6-Trimethylnaphthalene	–	460	1100	–	–	–	–	3700
1,6,7-Trimethylnaphthalene	–	–	–	–	–	–	–	1900
2,3,6-Trimethylnaphthalene	–	–	670	–	–	–	–	850
Decahydro naphthalene	–	–	–	–	–	800	–	190000

All concentrations are reported as estimated values, based on presumptive evidence of material.

–=Not detected at unspecified detection limit

Table D2 (continued): Summary of tentatively identified semivolatile organics analyses of soils from petroleum products sites #3 and #6 for the Soil Bioassay Pilot Study (ug/kg, dry).

Location	Western Washington Site #3			Eastern Washington Site #6				
Station Conc. Range	JC-1 Background	JC-2 Medium	JC-3 High	B-1 Background	B-2 Med(Gas)	B-3 Med(Diesel)	B-4 High(Gas)	B-5 High(Diesel)
2,3-dihydro-1,2-dimethyl-1H-indene	-	-	2300	-	-	-	-	-
2,3-dihydro-1,3-dimethyl-1H-indene	-	2500	-	-	-	-	-	-
2,3-dihydro-1,6-dimethyl-1H-indene	-	-	4100	-	-	-	-	-
2,3-dihydro-4,7-dimethyl-1H-indene	-	920	6500	-	-	-	-	-
2,3-dihydro-5,6-dimethyl-1H-indene	-	1400	14000	-	-	-	-	-
Octahydro-2-methyl-1H-indene	-	-	-	-	-	2000	-	-
1,2-Benzenedicarboxylic acid	-	-	-	-	240	-	-	-
Hexadecanoic acid	-	-	-	-	-	1900	-	-
Methyl decanoic acid	-	-	-	180	-	690	-	-
Methyl pentadecanoic acid	-	-	-	180	-	-	-	-
Methyl hexadecenoic acid	-	-	-	120	-	-	-	-
Tridecane	-	2900	-	-	-	-	-	-
Pentadecane	-	1000	-	-	-	-	-	-
Hexadecanal	-	-	-	-	-	2000	680	-
Octadecanal	-	-	-	5400	-	5000	-	-
4-Hydroxyl benzaldehyde	-	-	-	98	-	-	-	-
Unknown hydrocarbons	-	-	-	11000	11000	39000	3000	-

All concentrations are reported as estimated values, based on presumptive evidence of material.

-=Not detected at unspecified detection limit